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Antibiotic Susceptibility and Antimicrobial Activity of Lactic Acid Bacteria from Malaysian Fermented Foods Against Biofilm-Forming *Escherichia coli* Strains

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ABSTRACT

Lactic acid bacteria (LAB) are frequently employed in the food industry as food preservatives and starter cultures due to their potentially advantageous properties. Their ability to promote numerous health benefits in fermented foods has received increased attention. However, few studies have investigated the antibacterial activity of LAB and their susceptibility to antibiotics when combating biofilm-forming *Escherichia coli* strains. In this study, four fermented food samples were screened for presumptive LAB cultures, including Maman pickle (M5Bi), fermented fish (PB4iii), fermented durian (T6Aiii), and fermented glutinous rice (Ta2Ai), using the API 50 CHL method. The isolates were subsequently identified as *Lactobacillus plantarum, L. plantarum, Pediococcus pentosaceus*, and *Lactobacillus pentosus*, respectively, through molecular analysis of the 16S rDNA sequencing. The isolates were then assessed for antibiotic susceptibility to amoxicillin (25 µg), chloramphenicol (30 µg), tetracycline (30 µg), and erythromycin (10 µg) using the disc diffusion method. Moreover, the antibacterial activity of LAB against eight biofilm-forming *E. coli* strains was evaluated using the well diffusion method. The results showed that *L. plantarum* from the Maman pickle sample exhibited the most significant inhibitory zone against biofilm-forming *E. coli* strains. Importantly, all detected LAB were resistant to the four main antibiotics tested, indicating that the safety and efficacy of probiotics must be ensured before they can be developed and commercialized.

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1. Introduction

Lactic fermented foods have been consumed by humans since prehistoric times due to their long shelf lives and appealing organoleptic qualities. Today, the global market for fermented foods is growing and experiencing an upward trend [1]. Fermentation is a process in which carbohydrates are converted into alcohols or organic acids by bacteria or enzymes [2]. In Malaysia, fermented foods have gained popularity, with a variety of fermentation products being developed from fruits, vegetables, fish, milk products, and meats.

Recent studies have demonstrated that fermentation can enhance the physicochemical and microbiological quality of various food products, such as green roselle pickles, fermented glutinous rice ice cream, cow's milk and goat's milk *dadih*, fermented probiotic watermelon juice, fermented *Oreochromis niloticus*, and tempeh extract yogurt [3-8].

Customers have long been attracted to traditional fermented foods. Maman pickle, a lactic acid-producing pickled vegetable, offers a distinct flavour [9]. Another traditional food, fermented fish, is made by preserving fish through fermentation, typically combined with salt, herbs, spices, and vegetables to enhance flavour and texture during the process [10]. *Tempoyak*, a traditional Southeast Asian fermented durian flesh, is popular in Indonesia and Malaysia, often used as a seasoning or ingredient in various dishes [11]. *Tapai*, also known as fermented glutinous rice, is a traditional food prepared with starchy ingredients like glutinous rice, cassava or sweet potatoes. Traditionally, *tapai* is wrapped in a rubber leaf to enhance the smell and increase its palatability [12].

Lactic Acid Bacteria (LAB) are a class of coccus or rod-shaped, Gram-positive, non-spore forming bacteria that are well-known for their safety and frequent used in food due to their antimicrobial properties [11]. Traditional Asian fermented foods typically contain LAB considered as probiotics, such as *Lactobacillus plantarum, Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus casei, Leuconostoc mesenteroides, Lactobacillus kimchi, Lactobacillus fallax, Weissella confusa,* and *Weissella koreen* [13]. LAB significantly contribute to fermented food products by inhibiting the growth of pathogenic bacteria and preventing food spoilage [14].

Demir *et al.,* [15] found that the presence of LAB in foods not only preserves and enhances their nutritional value, but also improves their sensory qualities. LAB produce substances such as organic acids (like lactic acid), hydrogen peroxide, diacetyl, and bacteriocins, which can prolong the shelf life of food items and serve as bio-preservative agents in the food industry [11,16,17].

The study of foodborne bacteria and their susceptibility to antibiotics has attracted increasing interest [18,19]. Antibiotics are frequently added to food as additives to control food spoilage and treat bacterial infections [20]. However, it is important to recognize that microorganisms can develop antibiotic resistance, making it challenging to control their growth using traditional methods. As a result, it is essential to test LAB strains for antibiotic resistance before using them as probiotics in food. Previous study by Bahri *et al.,* [21] has reported that *E. coli* isolated from raw vegetables such as raw *ulam* has developed antibiotic resistance. In their study, *E. coli* isolates from raw ulam demonstrated a multiple antibiotic resistance index (MAR) ranging from 0.06 to 0.48.

Based on the available literature, it is evident that limited studies have focused on the ability of LAB isolated from Malaysian fermented foods to inhibit the growth of biofilm-forming *E. coli* strains. Consequently, the objectives of this study were to: (1) identify the presumptive LAB cultures isolated from Malaysian fermented foods using API 50 CHL and 16S rDNA sequencing; (ii) determine the antimicrobial activity against biofilm-forming *E. coli* strains using the well diffusion method; and (iii) assess the susceptibility of LAB strains to major antibiotics using disc diffusion method.

2. Methodology

The study involved identifying presumptive LAB from fermented foods using API 50 CHL for phenotypic identification and 16S rDNA sequencing for molecular identification. After identifying the four samples, they were tested for antibiotic susceptibility using the disc diffusion method and for antimicrobial activity against biofilm-forming *E. coli* strains using the well diffusion method.

2.1 Sources of Samples

LAB were isolated from four fermented food samples, namely fermented fish (*pekasam*) (P4Biii), fermented glutinous rice (*tapai*) (Ta2Ai), Maman pickle (M5Bi), and fermented durian flesh (*tempoyak*) (T6Aiii), which were purchased from a local night market in Kuantan, Malaysia. Approximately 25 g of each fermented food sample was mixed with 225 mL of buffered peptone water (Oxoid, UK) to obtain a 1:10 dilution. Serial dilutions were then prepared, and the diluted samples were spread onto de Man, Rogosa & Sharpe (MRS) agar. The plates were anaerobically incubated at 35°C for 48 hours. Colonies with distinct morphologies on the MRS agar plate were selected and sub-cultured by streaking on MRS agar to obtain a single colony. The stock cultures of LAB were maintained in MRS broth supplemented with 25% sterile glycerol and stored at −80 °C. Working cultures were prepared on slants of MRS agar and stored at 4°C. Prior to their use in experiments, the LAB cultures were transferred twice into the appropriate medium [5,6].

2.2 Phenotypic Identification Using API 50 CHL Method

The selected LAB strains from fermented foods were identified using the API 50 CHL to determine their phenotypic characteristics. Each isolated LAB was streaked onto MRS agar and incubated anaerobically at 35°C for 48 hours. A few colonies of LAB were then mixed evenly with approximately 5 mL of 0.85% saline water to obtain a $BaSO_4$ concentration equivalent to 2 McFarland, which corresponds to 6.0 $x10^8$ CFU/mL [22]. Each tube of the API 50 CHL strip was inoculated with the bacterial suspension using a sterile micropipette. The strips were placed in incubation trays with honeycombed wells, each filled with distilled or demineralized water according to the manufacturer's instructions. The strips were then incubated at 37°C for 48 hours. The reactions were visually inspected after 24-48 hours, and positive or negative results were determined based on colour changes in the tube due to anaerobic production of acid, detected by the pH indicator present in the chosen medium. The resulting biochemical profiles were identified and analysed in APIWEB™ to obtain the identity of the isolates and the percentage of similarity [23].

2.3 Genus Identification of LAB Using Polymerase Chain Reaction (PCR)

The four bacterial cultures identified using the API 50 CHL method were further analysed using Polymerase Chain Reaction (PCR) following method established by Bahri, [24]. Genomic DNA was extracted from 24-hour bacterial cultures using the innuPREP bacteria DNA kit (Analytik Jena), following the manufacturer's instruction. The extracted DNA was stored at -20°C until needed for PCR. The purity of the extracted DNA from all four samples was verified by agarose gel electrophoresis. To prepare the agarose gel, 0.8% agarose was dissolved in 1x TBE (Tris-borate-EDTA) buffer (pH 8.0), and 3 µl of SYBR safe DNA gel stain (Invitrogen, USA) was added to the gel before pouring it into the gel tray at a temperature of approximately 45-50°C. The gel wells were loaded with 10 µl of each extracted DNA sample with 3 µl of blue-orange loading dye (Promega, USA).

Electrophoresis was conducted at 75V for 40 minutes using a 10,000 bp DNA ladder as a marker. The genomic DNA was visualized under a blue-light LED transilluminator (JY-ERV-01, JUNYI, China), and the DNA ladder was used to determine the size of the genomic DNA.

The PCR reaction was performed using a gradient thermal cycler (MJ Mini Personal Thermal Cycler, Bio-Rad Laboratories, USA). Universal primers targeting the full-length 1.5 kb bacterial 16S rDNA were used, including the bacteria-specific primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and the universal primer 1492R (5'-GGTTACCTTGTTACGACTT-3') [25]. The total reaction mixture (25 µl) included gDNA purified using an in-house extraction method, 0.3 pmol of each primer, deoxynucleotides triphosphates (dNTPs, 400 µM each), 0.5 U DNA polymerase, PCR buffer, and water. The PCR protocol consisted of an initial denaturation step (94°C for 2 min), followed by 25 cycles of annealing and extension (98°C for 10 sec; 53°C for 30 sec; 68°C for 1 min). The PCR products were then purified using a standard method, and the purified products were subjected to bidirectional sequencing using universal sequencing primers 785F and 907R and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

2.4 16S rDNA Sequencing and Analysis

The purification and sequencing process was performed by Apical Scientific Sdn. Bhd. (Seri Kembangan, Selangor, Malaysia). The sequencing results were aligned using Molecular Evolutionary Genetic Analysis (MEGA) version 7. First, the reverse complement sequence from the reverse primer was aligned with the sequence from the forward primer to generate a consensus sequence. Next, the ClustalW2 multiple alignment programme was used to align the sequence. The sequence was then identified using the Basic Local Alignment Search Tool Nucleotide (BLASTn) against the GenBank database of the National Centre for Biotechnology Information (NCBI), with the setting of 16S ribosomal RNA gene sequences (Bacteria and Archaea) in the database and highly similar sequences (Megablast) were chosen. The top 10 sequences were selected and used to build the phylogenetic tree using the neighbour joining method [24].

2.5 Antibiotic Susceptibility of LAB Using Disc Diffusion Method

The susceptibility of the isolated LAB was tested using the method described by Ewnetu *et al.,* [26]. Four antibiotic discs, including amoxicillin (25 µg), chloramphenicol (30 µg), tetracycline (30 µg) and erythromycin (10 µg), were used to test the LAB strains for susceptibility towards antibiotics. Standardized culture suspensions with a concentration of 0.5 McFarland, equivalent to a $BaSO_4$ concentration of 1.5 x 10^8 CFU/mL, for each strain were swabbed onto Muller Hinton Agar (MHA) plates using a sterile cotton swab. Antibiotics discs were placed onto the MHA plates and incubated anaerobically for 24 h at 37 °C. The diameters of the inhibition zones were measured in millimetre (mm), and the results were recorded as sensitive (S) if the zone diameter was ≥ 21 mm, intermediate (I) if the zone diameter was 16-20 mm, or resistant (R) if the zone diameter was ≤ 15 mm, as described by Masood and Aslam [27].

2.6 Antimicrobial Activity Using Well Diffusion Method

To prepare for antimicrobial activity testing, 20 mL of MHA agar was poured onto each petri dish. Eight isolated *E. coli* cultures from raw ulam suspensions were swabbed onto the MHA plates using sterile cotton swabs. Prior to swabbing, each *E. coli* culture was suspended in saline water to a turbidity equivalent of 0.5 McFarland. The agar plates were allowed to dry for a few minutes at room

temperature (25 ± 2°C), and a sterile tip with a 7 mm diameter was used to punch a well into each agar plate. Each well was filled with 50 µl of LAB supernatant obtained from cultures grown in MRS broth medium. The cell-free supernatant from each LAB culture was prepared by centrifugation at 8000 x g for 20 minutes at 4°C, after which all LAB colonies and cells were harvested anaerobically at 30°C for 24 hours in MRS broth until the cultures reached approximately 10^7 CFU/ml. The cell-free supernatants were then used for antimicrobial activity testing using an agar well diffusion assay. The agar plates were allowed to diffuse at room temperature (25 ± 2°C) for 2 hours and then incubated in an upright position at 37°C for 24 hours. The results were determined by measuring the diameter of the growth inhibition zones in millimetres (mm) [24].

2.7 Statistical Analysis

The data analyses were performed in triplicate, and the results were reported as the mean value ± standard deviation (SD). To determine if there were any statistically significant differences among the averages, one-way analysis of variance (ANOVA) was performed using Microsoft Office Excel 2016 software and the statistical package Minitab version 14.0 software [28].

3. Results and Discussion

3.1 Phenotypic Identification of LAB Using API 50 CHL Kit

Phenotypic identification of LAB species was performed using the API 50 CHL kit. The results in Table 1 show that the isolate from Maman pickle was identified as *L. plantarum* with a high confidence level of 97.0%, while the isolate from fermented fish was identified as *L. plantarum* with a good confidence level of 94.1%. The isolate from fermented durian flesh was identified as *P. pentosaceus* with a confidence level of 96.5%, and the LAB isolate from fermented glutinous rice was identified as *L. pentosus* with an identification probability of 84.3%.

Table 1
Identification of LAB by using API 50CHL Kit

Samples	Codes of LAB strains	API 50 CHL	Confidence level	Similarity of Identification
Maman pickle	M5Bi	*Lactobacillus plantarum*	97.0% probability	Excellent
Fermented fish	P4Biii	*Lactobacillus plantarum*	94.1% probability	Good
Fermented durian flesh	T6Aiii	*Pediococcus pentosaceus*	96.5 % probability	Excellent
Fermented glutinous rice	Ta2Ai	*Lactobacillus pentosus*	84.3 % probability	Acceptable

Based on the results obtained, three out of the four LAB isolates were identified as belonging to the genus *Lactobacillus*, and one was identified as *Pediococcus*. Two isolates were identified as *L. plantarum.* According to Guidone *et al.,* [29], *L. plantarum* is a highly adaptable species that possesses many valuable properties and is commonly found in various fermented foods. Additionally, *L. plantarum* has been extensively used in the fermentation and manufacturing of fresh foods that are Generally Recognized As Safe (GRAS) and has a Qualified Presumption of Safety (QPS) status [30,31]. Devi *et al.,* [32] found that there are five different subspecies of the *L. plantarum*-group (LPG) in fermented vegetable products that can retain potential probiotic functionality. Liu *et al.,* [33]

supported the potential use of *L. plantarum* secondary metabolites from fermented products as a prospective probiotic for use in food processing to enhance the nutritive value of the food.

A study conducted by Rahayu and Endang [34] in Malaysia identified LAB strains from fermented fish as *L. plantarum,* while LAB strains from fermented glutinous rice were identified as *Lactobacillus* spp. Other studies, including Ida Muryany *et al.,* [35] has also identified LAB from fermented fish as *L. plantarum* with a confidence level of 99% similarity. In addition, studies by Endo *et al.,* [36] and Leisner *et al.,* [37] have shown that fermented durian flesh contains dominant LAB members of *L. plantarum* and *Fructobacillus durionis*. Adnan and Tan [38] identified LAB from fermented glutinous rice and fermented durian flesh in Malaysia as *Lactobacillus casei.*

3.2 Genus Identification of LAB Using 16S rDNA Sequencing

Figure 1 demonstrates the successful PCR amplification of the 16S rDNA gene in various LAB strains, resulting in a single band of approximately 1400-1500 bp for each strain. These LAB cultures (M5Bi, P4Biii, T6Aiii and Ta2Ai) were found to belong to *Lactobacillus* and *Pediococcus* genera, both of which are considered potential probiotics according to Porto *et al.,* [39]. In a previous study conducted by Jawan *et al.,* [40], LAB strains isolated from fermented glutinous rice and fermented durian flesh were identified as *Pediococcus acidilactici* and *Lactobacillus farciminis*, respectively. Phylogenetic tree analysis of these strains showed high bootstrap values in a range of 93% and 100%, which indicate a close relationship between the strains and their respective species [40].

Indri *et al.,* [41] identified *L. fermentum* as the bacterial species present in fermented durian flesh. Hagstrom *et al.,* [42] provided guidelines for determining the classification of isolates based on 16S rDNA sequence similarity, suggesting that similarities above 97% indicate the same species, while sequence similarities between 93% to 97% indicate different species within the same genus. In addition to these findings, Muryany *et al.,* [43] identified *L. plantarum* and *L. pentosus* as LAB species isolated from fermented fish through 16S rDNA sequence analysis. In another study, Sukirah *et al.,* [44] also identified *L. plantarum* as the LAB isolate present in *Maman pickle*, which is consistent with the result of this study.

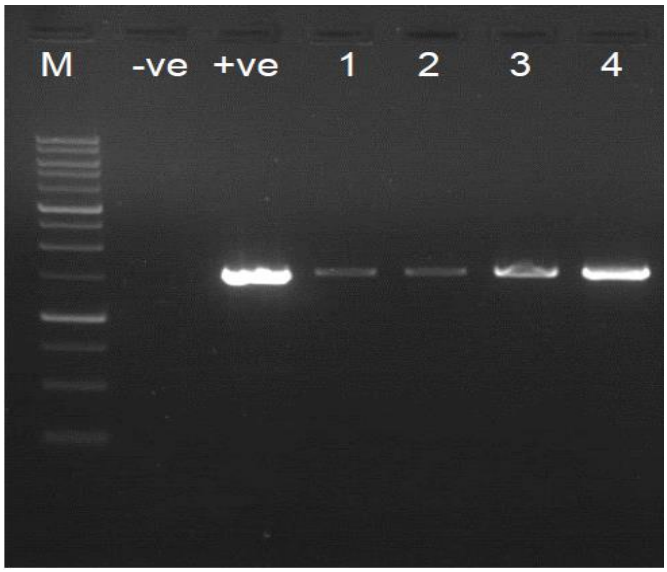
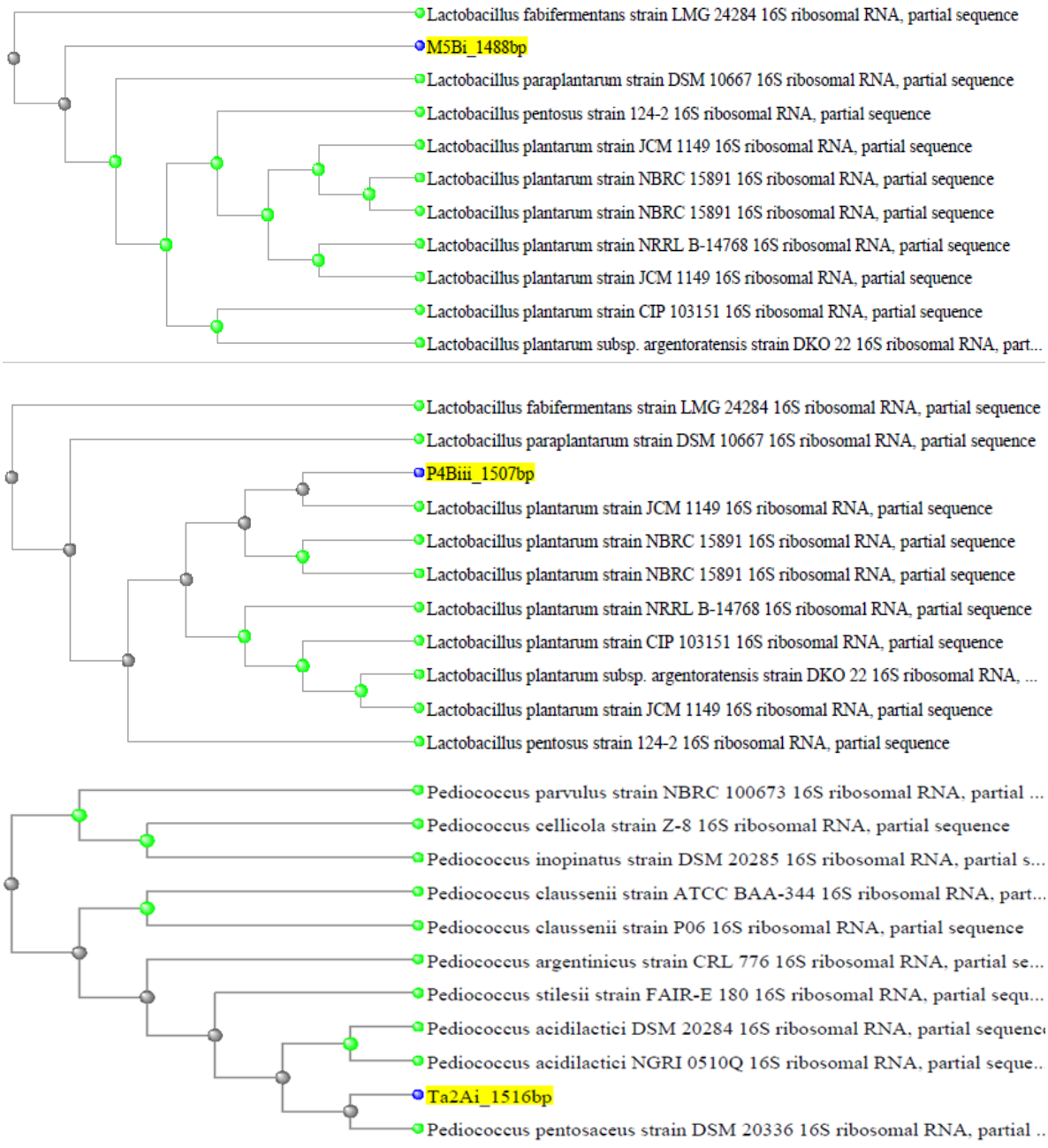


Fig. 1. Representative agarose gel analysis of PCR assay targeting 16S rDNA in LAB strains. Lane 1: DNA ladder, lane 2: negative control, lane 3: positive control, lane 4-7: positive sample

The construction of a phylogenetic tree using the LAB strains from this study and reference sequences from NCBI GenBank provides valuable insights into the taxonomic classification of these isolates. As shown in Figure 2, all isolates were closely clustered with the reference strains, suggesting that they are members of the Lactobacillaceae family. In particular, the M5Bi and P4Biii strains exhibited close nucleotide sequence similarity to *L. plantarum*, indicating that they likely belong to this species. Similarly, the T6Aiii and Ta2Ai strains were found to share the same clade with *P. pentosaceus*, suggesting that these strains also belong to that species. The results of this phylogenetic analysis further emphasize the diversity of LAB species present in various fermented foods and their potential as probiotics.



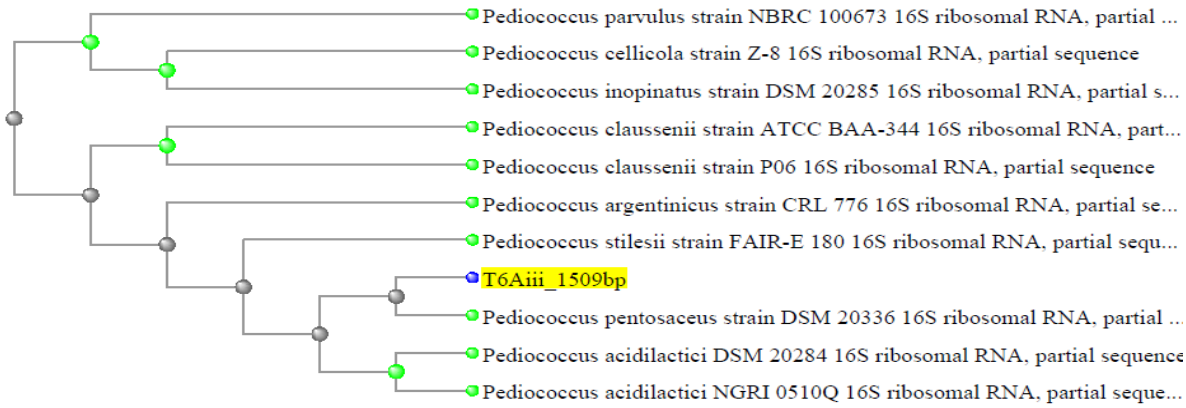


Fig. 2. Phylogenetic relationship of 16S rDNA sequence of each LAB strains. LAB obtained from NCBI database was included as reference strains

3.3 Antibiotic Susceptibility of LAB Isolates Using Disc Diffusion Method

Table 2 presents the antibiotic susceptibility profiles of LAB cultures isolated from fermented foods using the disc diffusion method. The results show inhibition zones of less than 11 mm for all isolates, indicating resistance to amoxicillin, chloramphenicol, tetracycline, and erythromycin. These findings align with those of Erginkaya *et al.,* [45], who reported that many LAB strains exhibit resistance to multiple antibiotics. This resistance is often attributed to the presence of bacteriocins, which are inhibitory compounds produced by bacteria during their growth.

However, the antibiotic resistance observed in these LAB isolates raises concerns about the potential transfer of resistance genes to pathogenic bacteria, which could pose a biohazard risk [46]. As a result, it is essential to thoroughly assess the safety of these LAB strains before considering their use as probiotics or in other applications within the food, pharmaceutical, or agricultural industries. Further studies should focus on characterizing the antibiotic resistance profiles of these isolates and investigating the potential for transfer of resistance genes to pathogenic bacteria.

Indeed, the major concern regarding antibiotic resistance is the overuse of antibiotics, which contributes to the development of drug-resistant bacteria. Flórez *et al.,* [47] found that Lactobacilli were generally susceptible to chloramphenicol and erythromycin, but their susceptibility to tetracycline varied depending on the species. The LAB strains in this study, specifically *L. plantarum*, *L. pentosus*, and *P. pentosaceus*, displayed resistance to the tested antibiotics, which may be due to the presence of antagonistic components such as p-amino benzoic acid (PABA) and thymidine [48]. This highlights the potential for LAB to mutate and develop into antibiotic-resistant bacteria (ARB) [49].

Table 2
Antibiotics susceptibility resistance profiles of four LAB strains isolated from fermented foods by disc diffusion

LAB cultures	Antibiotics	Total number of each LAB culture tested for antibiotic susceptibility (n=12)		
		Resistant (%)	Intermediate (%)	Susceptible (%)
L. plantarum (M5Bi)	Amoxicillin (25µg)	3/3 (100)	-	-
	Chloramphenicol (30µg)	3/3 (100)	-	-
	Tetracycline (30 µg)	3/3 (100)	-	-
	Erythromycin (10 µg)	3/3 (100)	-	-
L. plantarum (PB4iii)	Amoxicillin (25µg)	3/3 (100)	-	-
	Chloramphenicol (30µg)	3/3 (100)	-	-
	Tetracycline (30 µg)	3/3 (100)	-	-
	Erythromycin (10 µg)	3/3 (100)	-	-
P. pentosaceus (T6Aiii)	Amoxicillin (25µg)	3/3 (100)	-	-
	Chloramphenicol (30µg)	3/3 (100)	-	-
	Tetracycline (30 µg)	3/3 (100)	-	-
	Erythromycin (10 µg)	3/3 (100)	-	-
L. pentosus (Ta2Ai)	Amoxicillin (25µg)	3/3 (100)	-	-
	Chloramphenicol (30µg)	3/3 (100)	-	-
	Tetracycline (30 µg)	3/3 (100)	-	-
	Erythromycin (10 µg)	3/3 (100)	-	-

**Resistant (≤11), Intermediate (12-18), Susceptible (≥19)*
***Clinical and Laboratory Standards Institute (CLSI) [50]*

3.4 Antimicrobial Activity of LAB Using Well Diffusion Method

E. coli is a pathogenic bacterium that is frequently found in environments, foods, water, and humans. Despite the presence of many microbial species capable of forming biofilms, *E. coli* forms biofilms and is a common cause of contaminated food. According to a study by Bahri *et al.,* [21], fresh vegetable biofilms produced by *Salmonella* and *E. coli* isolates can act as sources of bacterial transmission in the food chain. To prevent or stop the growth of *E. coli* biofilm, various techniques, including physical, chemical, biological, and synergistic methods, have been explored and implemented [51]. According to Cizeikiene *et al.,* [52], one of these methods involves the use of antimicrobial compounds produced by specific LAB that can effectively control or stop the growth of harmful bacteria, striving to improve the safety of food products.

The isolated LAB strains were screened for antimicrobial activity against selected *E. coli* strains previously isolated from ulam-ulam, a Malaysian salad, in a study by Anis Athirah [24]. These *E. coli*

strains were known to form biofilms. The well diffusion method was employed to assess this activity. Table 3 presents the results, which indicate clear zones around the wells due to the metabolites produced by the isolated LAB from fermented foods. These metabolites effectively inhibited the activities of the biofilm-forming *E. coli* strains.

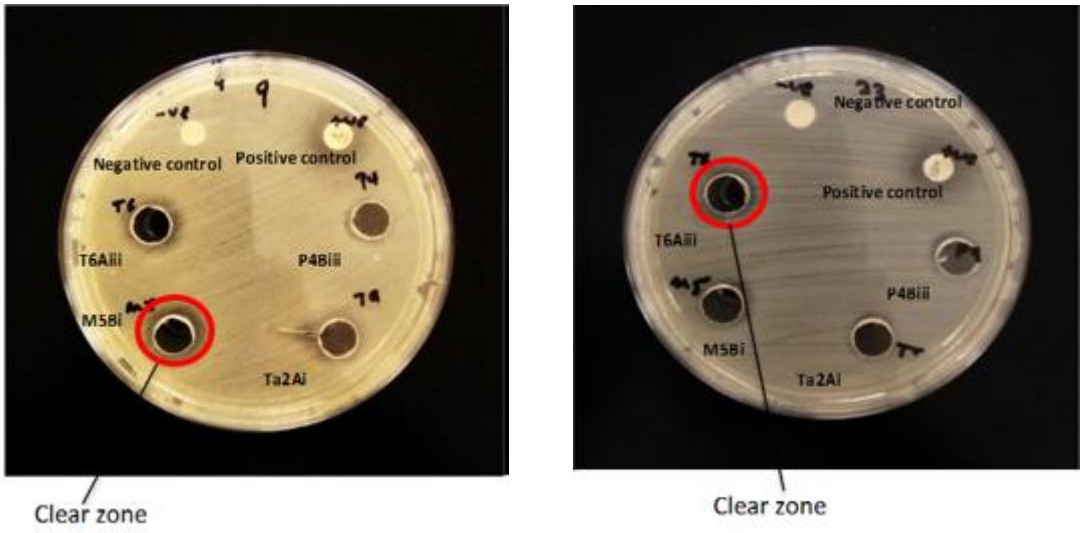
Table 3
Inhibitory action of different LAB strains against *E. coli* strains forming biofilm

E. coli isolates	Types of Ulam	*L. plantarum* (M5Bi)	*L. plantarum* (P4Biii)	*P. pentosaceus* (T6Aiii)	*L. pentosus* (Ta2Ai)
E. coli (SMKB2)	*Ketumbar*	12.00 ± 2.00^{a}	10.70 ± 1.20^{a}	10.70 ± 1.20^{a}	10.70 ± 1.20^{a}
E. coli (SMKB8)	*Ketumbar* (SMKB8)	10.00 ± 0.00^{ab}	11.33 ± 1.20^{a}	9.33 ± 1.20^{b}	10.70 ± 1.20^{ab}
E. coli (SMK2)	*Kesum*	10.70 ± 1.20^{a}	10.00 ± 0.00^{a}	12.00 ± 2.00^{a}	12.00 ± 2.00^{a}
E. coli (SMK3)	*Kesum*	10.00 ± 2.00^{a}	0.00^{b}	9.33 ± 1.20^{a}	0.00^{b}
E. coli (SMU2)	*Ulam raja*	9.33 ± 1.20^{b}	12.00 ± 2.00^{a}	10.70 ± 1.20^{ab}	10.70 ± 1.20^{ab}
E. coli (SMT4)	*Taugeh*	10.00 ± 2.00^{a}	0.00^{b}	10.70 ± 1.20^{a}	0.00^{b}
E. coli (WMDS)	*Daun sup*	10.00 ± 2.00^{a}	0.00^{b}	10.70 ± 1.20^{a}	0.00^{b}
E. coli (WMPP2)	*Pucuk putat*	12.70 ± 1.20^{a}	10.70 ± 1.20^{b}	11.33 ± 1.20^{ab}	10.00 ± 0.00^{b}

**Values are mean ± SD of four LAB strains for each E. coli strains forming biofilm, where p<0.05 has significant difference. (0.00) indicates no inhibition zone.*
**SM: Supermarket; WM: Wet market; KB: Ketumbar; K: Kesum; U: Ulam Raja; T: Tauge; DS: Daun sup; PP: Pucuk Putat*
**M5Bi (Maman pickles), P4Biii (fermented fish), T6Aiii (fermented durian flesh), Ta2Ai (fermented glutinous rice)*

Figure 3 illustrates the inhibition zones of LAB antimicrobial activity against biofilm-forming *E. coli* isolates. The antimicrobial properties of LAB strains, such as *L. plantarum*, have been reported in previous studies. For example, Francois *et al.,* [53] found that *L. plantarum* exhibited antimicrobial activity against *Klebsiella pneumoniae, Pseudomonas* sp., and *Enterococcus faecalis*. Similarly, Rao *et al.,* [54] reported significant antimicrobial activity against *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus*, and other bacterial strains by various strains of *L. pentosus* and *L. plantarum*. Based on the literature, the production of pediocin, a bacteriocin produced by *Pediococcus*, may be responsible for its antilisterial activity in fermented sausage or salami, as documented in several studies [55,56].

These findings suggest that LAB strains, including those isolated in this study, possess promising antimicrobial properties that could be harnessed for potential applications in food preservation, pharmaceuticals, or agriculture. However, further research is needed to characterize the antimicrobial compounds produced by these LAB strains and to evaluate their safety and effectiveness in various applications.



(a) (b)

Fig. 3. Inhibition zone of LAB against *E. coli* strains forming biofilm due to the antimicrobial activity (a) Plate no.9 was the sample of SMKB2 (*Ketumbar)*, (b) Plate no.23 was the sample WMPP2 (*Pucuk putat*).

Hajime *et al.,* [57] established criteria for evaluating the antimicrobial activity of isolates, where clear zones of less than 9 mm indicate weak activity, and diameters greater than 12 mm suggest strong antimicrobial activity. Based on these criteria, the isolates from fermented foods in this study exhibited varying antimicrobial activities. *L. plantarum* (M5Bi) displayed the highest antimicrobial activity against *E. coli* (WMPP2), while *L. plantarum* (P4Biii) and *L. pentosus* (Ta2Ai) showed no inhibition zone against *E. coli* (SMK3, SMT4, and WMDS).

The inhibitory action of LAB strains against pathogenic bacteria can be attributed to the production of H_2O_2, bacteriocins, and organic acids during their growth [58]. The organic acids in LAB metabolites exert their antimicrobial effects by acting on the cytoplasmic membrane, neutralizing its electrochemical potential, and increasing its permeability. This action results in bacteriostatic effects and ultimately leads to the death of susceptible bacteria [59].

The antimicrobial properties of probiotics may be attributed to the production of bacteriocins, such as nisin [60]. Nisin, the first approved bacteriocin for use in the food industry, is commercially produced using a culture of *Lactococcus lactis* and is effective against numerous Gram-positive bacteria [61]. It has also demonstrated effectiveness against certain Gram-negative spoilage organisms and pathogens, including *E. coli, Pseudomonas aeruginosa*, and *Salmonella* Typhimurium [62].

These findings highlight the potential of LAB strains to serve as antimicrobial agents in various applications, such as food preservation, pharmaceuticals, or agriculture. However, further research is needed to characterize the specific antimicrobial compounds produced by these LAB strains and to assess their safety and efficacy in different contexts. LAB indeed play a crucial role in preserving and ensuring the safety of fermented foods, contributing to the microbial stability of the final product. However, as this study discovered, all LAB isolates exhibited resistance to four major antibiotics—amoxicillin, chloramphenicol, tetracycline, and erythromycin. While antibiotic resistance in LAB does not automatically disqualify them as probiotics, it does raise concerns.

Probiotics are live microorganisms that provide health benefits to the host when consumed in adequate amounts. When a LAB strain exhibits antibiotic resistance, it is essential to investigate the reasons behind this development. In some instances, the resistance may be due to natural genetic variability or exposure to low levels of antibiotics in the environment, which may not necessarily pose

a risk to human health. However, if the antibiotic resistance has emerged from the use of antibiotics in animal feed or agricultural practices, this issue becomes more concerning [63,64].

Given these considerations, it is crucial to thoroughly evaluate the safety of LAB strains with antibiotic resistance before considering their use as probiotics or for other applications within the food, pharmaceutical, or agricultural industries. Further research should focus on understanding the mechanisms of antibiotic resistance in LAB and assessing the potential risks to human health associated with the use of antibiotic-resistant LAB strains.

4. Conclusions

This study has demonstrated that four LAB strains from fermented foods were identified through API 50 CHL and 16S rDNA sequencing. Some strains exhibited antimicrobial activity against biofilm-producing *E. coli s*trains, however, all strains were resistant to major antibiotics tested, including amoxicillin, chloramphenicol, tetracycline, and erythromycin. In conclusion, the study revealed that each LAB isolate had varying degrees of efficacy in inhibiting biofilm-forming *E. coli,* with *L. plantarum* isolated from *Maman pickle* demonstrating the highest inhibition zone against eight *E. coli* isolates. It is crucial to consider the safety and efficacy of specific LAB strains when selecting probiotics. Strains with extensive research and proven safety and efficacy records are recommended. Further research and development of probiotics necessitate rigorous investigation to ensure compliance with authorized health guidelines before they are ready to be commercialised.

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