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Potential Use of *Lactiplantibacillus plantarum BCC 4352* as a Functional Starter Culture for Fermenting Thai Pork Sausage (Nham)

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Abstract: The suitability of *Lactiplantibacillus plantarum* (*L. plantarum*) as a functional starter culture in Nham fermentation was investigated, with a focus on evaluating both its probiotic attributes and fermentation capability. *L. plantarum* BCC 4352 (*Lpb*BCC4352) exhibited colony-associated antimicrobial activity against *Kocuria rhizophila*, *L. plantarum*, *Latilactobacillus sakei* ssp. *sakei*, and *Pediococcus pentosaceus*, as well as the zoonotic *Streptococcus suis*. *Lpb*BCC4352 exhibited impressive acid (pH 2.5) and bile resistance, coupled with notable survival rates in a simulated human digestive model. In addition, the strain is able to utilize fructo-oligosaccharides in simulated human colon conditions. It also displayed robust adhesion to human colon cell monolayers (Caco-2) and gastric mucin. Furthermore, it showed a promising cholesterol reduction ability in the fermentation medium. The safety of *Lpb*BCC4352 for human consumption was confirmed through a hemolytic activity assay and antibiotic susceptibility testing. Moreover, using *Lpb*BCC4352 as a starter culture not only enhanced the firmness of Nham but also ensured consumer satisfaction. The overall findings emphasize the potential use of *Lpb*BCC4352 as a safe and effective functional starter culture, particularly in the production of Nham.

Keywords: Nham; *Lactiplantibacillus plantarum*; probiotic; starter culture; fermented pork sausage; functional starter

1. Introduction

Probiotics, as defined by The International Scientific Association for Probiotics and Prebiotics (ISAPP), are "live microorganisms that, when administered in adequate amount, confer a health benefit on the host" [1]. The criteria for assessing probiotic microorganisms intended for use in foods emphasize not only their capability to withstand the harsh conditions of the digestive tract, including resistance to gastric juices and bile, but also the importance of their ability to thrive in the gut [2]. This underscores the necessity of incorporating them into a suitable food vehicle. This incorporation is intended to enhance resilience during passage through the acidic stomach environment, increase resistance to elevated

bile salts concentrations, and facilitate the eventual colonization in the intestinal tract [3]. In addition to other criteria, such as reducing cholesterol levels [4] and utilizing prebiotic carbohydrates [5], safety factors such as potential pathogenicity to humans or animals, including hemolytic activities [6], and antibiotic resistance have also been highlighted.

Several bacterial genera have been suggested as probiotics; nevertheless, the most employed probiotic bacteria are lactic acid bacteria (LAB) [7–9]. Among LAB strains, *Lactobacillus plantarum*, which has been reclassified as *Lactiplantibacillus plantarum* [10], is widely recognized as a probiotic with a well-established safety record. This recognition is endorsed by various studies [11–13] and validated by the European Food Safety Authority (EFSA) in 2012 [14]. Nevertheless, sporadic reports of opportunistic infections linked to these bacteria have emerged [15].

L. plantarum plays a significant role in fermenting a wide range of products, including dairy products [16–18], fermented meat products [19–22], and various plant-based products [23–25]. Numerous investigations have been conducted to develop functional foods or preparations that incorporate probiotics, such as fermented sausages [26–34]. This product is particularly attractive because it remains unheated and maintains high populations of LAB. Moreover, its matrix appears to function as a protective shield, enhancing the survival of probiotic strains through the gastrointestinal tract [35]. In the current study, we considered Nham or Thai fermented pork sausage as an ideal carrier for probiotic cultures due to its consistent fermentation by a dominant microorganism known as lactobacilli [19,36].

Nham, a semi-dry fermented raw pork product, is widely cherished by the Thai population due to its distinct qualities, such as its firm texture, sour flavor, and vibrant red color. These unique characteristics primarily result from the specific ingredients and microorganisms employed during the fermentation process, as indicated by Valyasevi and Rolle [19] and Visessanguan et al. [20]. Nham is typically crafted from a blend of minced raw pork, cooked pork rind, cooked rice, fresh garlic, whole fresh bird's eye chili, and curing salts. This mixture is tightly packed into plastic casings or banana leaves and left to ferment at an ambient temperature of 30–35 °C for approximately 5 days until the pH level drops below 4.6 [37].

In Thailand, there is a proposed initiative for the incorporation of probiotics into Nham. Phupaboon and colleagues [38] conducted a study in which they illustrated an in vitro probiotic assessment, evaluating the resistance to pH and bile salts of the strains both before and after encapsulation. Furthermore, they employed potent probiotics to ferment a laboratory-scaled Nham by encapsulating probiotic starter cultures.

The present study emphasizes the potential transformation of traditional Thai fermented pork into an innovative sausage product, aiming to enhance its market appeal and nutritional content. Our objective is to develop probiotic enriched Nham. The investigation explores the probiotic characteristics of selected *L. plantarum* strains, evaluating both their functionality and safety. Additionally, we highlight their suitability as a starter culture for Nham fermentation in a validated industrial-scale production of this kind of fermented pork sausage.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

L. plantarum BCC 4352, previously isolated from Nham samples, was obtained from the BIOTEC culture collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC) (Khlong Luang, Thailand). The probiotic *L. plantarum* NCIMB 8826 (*Lpb*NCIMB8826), employed as the positive control in the experiment, was sourced from The National Collection of Industrial Food and Marine Bacteria (NCIMB) (Scotland, UK). All LAB strains were stored at −80 °C in deMan, Rogosa, and Sharpe (MRS) medium with 15% glycerol (Merck, Germany) and propagated in MRS broth at 30 °C for 18 h before being used for testing of their antimicrobial production.

Both Gram-positive and Gram-negative bacteria, as listed in Table 1, were used as bacterial indicators to determine the antimicrobial activity of *L. plantarum*. *Staphylococcus*

aureus, *Listeria innocua*, *Escherichia coli*, and *Salmonella* Typhimurium were cultured in tryptic soy broth (TSB, Merck, Germany), supplemented with 0.6% yeast extract, and incubated at 37 °C for 18 h with shaking at 200 rpm. *Streptococcus suis* serotype 2 was cultured in Todd–Hewitt broth with 2% yeast extract at 30 °C for 18 h. The remaining LAB indicator strains were propagated in MRS medium at 30 °C for 18 h.

Table 1. Antagonistic activity of *L. plantarum* BCC4352 (*Lpb*BCC4352) against wide ranges of bacterial indicators.

Indicator Strains	Antagonistic Activity	
	Cell-to-Cells	Cells Free Supernatant (CFS)
Gram-positive bacteria		
Bacillus circulans JCM 2504^T	-	-
Bacillus coagulans JCM 2257^T	-	-
Bacillus subtilis ssp. *subtilis* JCM 1465^T	-	-
Enterococcus faecalis JCM 5803^T	-	-
Enterococcus faecium JCM 5804^T	-	-
Kocuria rhizophila NBRC 12708	+	-
Lactiplantibacillus plantarum ATCC 14917^T	+	-
Lactiplantibacillus plantarum NCIMB 8826	-	-
Latilactobacillus sakei ssp. *sakei* JCM 1157^T	+	-
Lactococcus garvieae BCC 43578	-	-
Leuconostoc mescenteroides ssp. *mes* JCM. 6124^T	-	-
Listeria innocua ATCC 33090^T	-	-
Pediococcus dextrinicus JCM 5887^T	-	-
Pediococcus pentosaceus JCM 5885	+	-
Staphylococcus aureus ATCC 23235	-	-
Staphylococcus aureus ATCC 25923	-	-
Staphylococcus aureus ATCC 6512	-	-
Staphylococcus aureus ATCC 29213	-	-
Streptococcus gordonii DMST 35778	-	-
Streptococcus mutan DMST 18777	-	-
Streptococcus pyrogenes DMST 17020	-	-
Streptococcus suis P1/7 *	+	-
Weissella paramesenteroides JCM 9890^T	-	-
Propionibacterium acnes ATCC 11827	-	-
Propionibacterium acnes DSM 1897	-	-
Propionibacterium acnes DSM 16379	-	-
Propionibacterium acnes JCM 6473	-	-
Gram-negative bacteria		
Escherichia coli ATCC 25922	-	-
Escherichia coli O157:H7 ATCC 43888	-	-
Salmonella Typhimurium ATCC 13311	-	-

Remarks; "+": the strain exhibited a clear zone of inhibition against the tested bacterium; "-": the strain did not exhibit a clear zone of inhibition against the tested bacterium. ATCC, American Type Culture Collection, Rockville, MD; BCC, BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand; JCM, Japan Collection of Microorganisms, Saitama, Japan; NBRC, NITE Biological Resource Center, Chiba, Japan; NCIMB, National Collection of Industrial, Marine, and Food Bacteria, Scotland, UK. DMST, Department of Medical Sciences Thailand culture collection. * The Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences Thammasat University, Pathum Thani, Thailand.

2.2. Determination of Antimicrobial Activity

The antimicrobial activity of *L. plantarum* strains was assessed using the spot-on-lawn technique as described by Zendo et al. [39]. In assessing the antimicrobial activity of *L. plantarum*, a 10 µL aliquot of an overnight culture of *L. plantarum* (8 log CFU/mL) was inoculated onto an indicator plate pre-seeded with 50 µL of the bacterial indicator (7 log CFU/mL). The medium used for each indicator was the same as described in Section 2.1 and supplemented with 1.5% agar. Following a 30-min drying period, the plates

were incubated at 30 °C for 24 h. The presence of a clear zone surrounding the culture spots indicated the antagonistic activity of *L. plantarum* against the bacterial indicator strains.

The antimicrobial activity of the cell-free supernatant (CFS) was evaluated by collecting the CFS samples from the tested strains through centrifugation (8500× *g* for 10 min at 4 °C), followed by adjusting the pH to 6.5. The neutralized CFS was subsequently sterilized by filtering through a 0.2 µm sterile cellulose acetate filter (ADVANTEC®, Tokyo, Japan) and was then subjected to two-fold serial dilution with sterilized distilled water. A 10 µL of each dilution were applied to agar plates that had been previously overlaid with 5 mL of soft agar (1% agar) containing approximately 7 log CFU/mL of the indicator strain. The medium used for each indicator was the same as described in Section 2.1 and supplemented with 1.5% agar. Plates were then incubated at 30 °C for 24 h. The antimicrobial activity of the sample was expressed in arbitrary units (AU) per milliliter (mL), with one AU defined as the reciprocal of the highest dilution at which the growth inhibition remained detectable.

2.3. Determination of Acid and Bile Tolerance

The tolerance of *Lpb*BCC4352 cells to stress conditions, including low pH and bile salts, was assessed based on the method outlined by Vijayakumar et al. [40]. To evaluate acid tolerance, a 1 mL aliquot of an overnight culture of *Lpb*BCC4352 (9 log CFU/mL) was inoculated into 9 mL of acidified MRS broth (pH 2.5). After 2 h of incubation at 37 °C, viable *Lpb*BCC4352 cells grown under acidic conditions were enumerated and expressed as logarithms of colony-forming units per milliliter (log CFU/mL). The survival rates were calculated as follows:

$$\text{Survival rate (\%)} = (C_1/C_0) \times 100\%$$

where C_1 is the viable count after acid treatment (log CFU/mL), and C_0 denotes the initial viable count (log CFU/mL).

For the bile tolerance test, 1 mL of *Lpb*BCC4352 cells with a concentration of 9 log CFU/mL, was introduced into 9 mL of MRS broth supplemented with varying amounts of oxgall from Sigma-Aldrich (0, 0.1, 0.3, 0.5, 0.7, 1.0, and 2.0% *w*/*v*), and the mixture was then incubated at 37 °C for 4 h. The cell viability was measured by standard plating methods and expressed in log CFU/mL. Bile tolerance, expressed as a survival rate of the tested strain after bile salt treatments, were determined using the previously mentioned formula for acid tolerance, where C_1 represents the viable count after oxgall treatment, and C_0 indicates the initial viable count.

2.4. Resistance to the Simulated Digestion Conditions

The survivability of *Lpb*BCC4352 cells in the human digestive system was assessed using the simulated digestive model established by Minekus et al. [41] with slight modifications. The simulation comprised sequential oral, gastric, and intestinal phases. An overnight culture of *Lpb*BCC4352 was harvested via centrifugation (10,000× *g* for 10 min at 4 °C) and subsequently washed twice and resuspended with saline solution. The culture suspension, comprising 1 mL aliquots with a bacterial concentration of approximately 9 log CFU/mL, was introduced into 9 mL of simulated salivary fluid (SSF, pH 7) containing amylase enzyme (75 U/mL, pH 7). The mixture was then incubated at 37 °C for 2 min. During the simulated gastric phase, samples from the oral phase were combined with 10 mL simulated gastric fluid (SGF, pH 3) supplemented with pepsin (2000 U/mL, pH 3) and subsequently incubated dynamically at 37 °C for 2 h using a Trayster vertical rotating mixer (IKA, Guangzhou, China) operating at 5 rpm/min. During the simulated intestinal phase, samples from the gastric phase were mixed with 20 mL of simulated intestinal fluid (SIF, pH 7) enriched with bile acid (10 mM) and pancreatin (including trypsin enzyme at 100 U/mL, pH 7). Afterward, the sample was subjected to a 4-h dynamic incubation (5 rpm/min) in an anaerobic chamber (DG500, Whitley Ltd., Surrey, UK) at 37 °C, maintaining anaerobic conditions with 5% carbon dioxide, 5% helium, and 90% nitrogen. Samples were collected at different time intervals, and the viability of *Lpb*BCC4352 cells was evaluated through standard plating on MRS agar plates.

2.5. Detection of the Bile Salt Hydrolase Activity

The qualitative direct plate assay for evaluating the bile salt hydrolase activity of *Lpb*BCC4352 was performed following the procedure described by Allain et al. [42] with slight modifications. In brief, *Lpb*BCC4352 was streaked onto MRS plates containing 0.5% (*w*/*v*) taurine conjugates of deoxycholic acid (TDCA) or glycocholic acid conjugates of deoxycholic acid (GDCA), supplemented with 0.37 g of $CaCl_2$ per liter. The plates were subsequently incubated anaerobically at 37 °C for 72 h in an anaerobic chamber, with a gas mixture comprising 5% carbon dioxide, 5% helium, and 90% nitrogen. Control plates of MRS agar medium lacking TDCA or GDCA supplementation were prepared. A positive reaction was determined by the presence of precipitated bile acid or the formation of an opaque halo around the colonies.

2.6. Adhesion Assay

2.6.1. Adhesion to the Human Colon Adenocarcinoma Cell Line (Caco-2)

The adhesion capability of *Lpb*BCC4352 to the human colon adenocarcinoma cell line (Caco-2) was evaluated according to the method of Klingberg et al. [43]. The adhesion assay was performed in a 24-well tissue culture plate (Costar® 3524, Wiesbaden, Germany) with 10-day-old monolayers of Caco-2 cells (4 log cells/well). After rinsing the cells twice with phosphate buffered saline (PBS, pH 7.2), they were then immersed in 2 mL of non-supplemented Modified Eagle's Medium (MEM) at 37 °C for 1 h. After the incubation, the MEM was replaced with a 200 µL suspension of *Lpb*BCC4352 cells in MEM at a concentration of 7 log CFU/mL. The cell adhesion experiment was performed at 37 °C for 1 h under 5% carbon dioxide and 95% humidity. Following incubation, the monolayer cells treated with bacteria were washed 10 times with sterile PBS solution to eliminate any unattached bacterial cells. Subsequently, 0.05% (*v*/*v*) Triton X-100 was introduced into the wells for 10 min to detach the Caco-2 cells along with the adhered bacteria. The quantification of viable adhering bacteria was accomplished by plating serial 10-fold dilutions of the solution containing the detached Caco-2 cells and intact bacterial cells onto MRS-CaCO3 agar, followed by a 48-h incubation at 37 °C. The capability of bacteria to adhere to the Caco-2 cell layer was determined using the following equation:

$$\text{Adhesion (\%)} = (X_t / X_0) \times 100$$

where, X_0 represents the initial number of bacteria added to each well, and X_t represents the number of bacteria after detachment with Triton X-100.

2.6.2. Mucin Adhesion Assay

Mucin adhesion experiments were adapted from Van den Abbeele et al. [44]. The mucin adhesion assay was performed in 96-well polystyrene microtiter plates (Nunc MaxiSorp, Thermo Fisher Scientific, Waltham, MA, USA), using porcine gastric mucin (Sigma-Aldrich®, St. Louis, MO, USA) as a matrix. The wells were coated with 200 µL of mucin solution (1 mg/mL) in PBS, pH 7.0 and incubated at 4 °C for 24 h. Following incubation, the wells were washed twice with 200 µL of PBS to eliminate unbound mucin, and then 200 µL of *Lpb*BCC4352 cell suspension (9 log CFU/mL) was introduced into the wells. Plates were further incubated at 37 °C for 30 min. After the incubation, wells were washed in triplicate with 200 µL of sterile PBS to remove unbound bacteria. Another 300 µL of 0.5% (*v*/*v*) Triton X-100 were added and incubated at 37 °C for 30 min to detach the bacterial cells from wells. The number of adhesive bacterial cells was assessed through the standard plating method on MRS agar and expressed as log CFU/well. The capability of bacteria to adhere to the mucin layer was determined using the following equation:

$$\text{Adhesion (\%)} = (X_t / X_0) \times 100$$

where, X_0 represents the initial number of bacteria added to each well, and X_t represents the number of bacteria after detachment with Triton X-100.

2.6.3. Detection of Binding-Related Genes

DNA was extracted from an overnight culture of *Lpb*BCC4352 and *Lpb*NCIMB8826 using the Wizard genomic DNA purification kit (Promega, Charbonnieres, France). PCR was conducted to detect the presence of genes associated with the binding mechanisms to epithelial cells, employing specific primers designed by Turpin et al. [45], using the primer sequences provided in Table S1. The PCR conditions comprised one cycle at 95 °C for 5 min, followed by 40 cycles at 95 °C for 30 s, 54 °C for 15 s, and 72 °C for 30 s, concluding with a final step at 72 °C for 5 min. Verification of the presence of specific PCR products was performed on a 2.0% (*w*/*v*) agarose gel.

2.7. Growth of LpbBCC4352 in Prebiotic Medium

The prebiotic oligosaccharide, FOS (fructo-oligosaccharides, F8052), used in this study, was purchased from Sigma-Aldrich®. The growth capacity of *Lpb*BCC4352 in the presence of tested carbohydrates was assessed using the modified standard ileal efflux medium (mSIEM). This medium, designed to simulate the nutritional characteristics of the colon by Gibson et al. [46], was employed in the test following the protocol outlined by Gamonpilas et al. [47] with minor modification. A 0.5 mL culture of *Lpb*BCC4352 cells, containing a cell concentration of 9 log CFU/mL, was cultured in MRS broth for 24 h, followed by centrifugation at 8500× *g* for 10 min at 4 °C to harvest the cells. The resulting cell pellets were washed twice with 50 mM phosphate-buffered saline (pH 7.2) and then resuspended in 4.5 mL of O_2-free basal mSIEM medium without the addition of any carbohydrates. A 0.1 mL aliquot of the culture suspension was introduced into 0.9 mL of the mSIEM medium, which was prepared without glucose and included either 2% (*w*/*w*) FOS or control carbohydrates (glucose). Samples were then incubated at 37 °C for 48 h in an anaerobic chamber with a gas mixture consisting of 5% carbon dioxide, 5% helium, and 90% nitrogen. The ability of *Lpb*BCC4352 to utilize the tested carbohydrates was assessed through the analysis of viable cell growth (log CFU/mL), pH changes, and acid production.

2.8. Analysis of Short Chain Fatty Acids (SCFAs)

Analysis of short-chain fatty acids produced by *Lpb*BCC4352 was conducted by modifying the method of Schooley et al. [48]. A 500 µL of the culture supernatant of *Lpb*BCC4352 from Section 2.7 was mixed with trans-cinnamic acid at a concentration of 0.5 mM (internal standard). Subsequently, 100 µL of hydrochloric acid (HCl) and 2 mL of diethyl ether were added to the vial. The mixture was then mixed using a multichannel vortex apparatus at a speed of 2500 rpm for 5 min. Afterward, the sample was centrifuged at 200× *g* for 5 min, and the upper layer was withdrawn to a volume of 500 µL. Derivatization was performed using N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-Butyldimethyl chlorosilane (MTBSTFA + 1%TMCS) at a volume of 50 µL under a temperature of 80 °C for 40 min. Following derivatization, the sample was analyzed for short-chain fatty acids (SCFAs) using a GC-MS instrument in selected ion monitoring (SIM mode), employing a DB1 column and utilizing standard substances to construct a calibration curve for determining the concentration of the target compounds in the sample.

2.9. Cholesterol Assimilation Assay

The experiment was performed based on the method of Liong & Shah [49]. A water-soluble cholesterol (cholesterol-polyethylene glycol 600, Sigma-Aldrich) was added to the sterile MRS broth supplemented with 0.3% oxgall to make a final concentration of 20 µg/mL. An overnight culture of *Lpb*BCC4352 cells (9 log CFU/mL) was then introduced into the broth, and the mixture underwent anaerobic incubation at 37 °C for 72 h using an anaerobic chamber (DG500, Whitley Ltd., UK). Following the incubation period, *Lpb*BCC4352 cell viability was measured by plating methods. The o-phthalaldehyde method, as described by Rudel & Morris [50], was employed to quantify the residual cholesterol levels in the collected cell-free supernatant. In brief, the cell-free supernatant was mixed with 33% (*w*/*v*) potassium hydroxide (KOH) and absolute ethanol. The resulting mixture was heated to

60 °C for 15 min, followed by cooling to ambient temperature. After cooling, hexane was added, leading to phase separation. The hexane phase was then collected, transferred to a glass tube, and evaporated under a nitrogen gas flow. Once dried, the remaining residue was treated with the o-phthalaldehyde reagent and subsequently mixed with 98% sulfuric acid. After a 10-min incubation at 30 °C, the absorbance was read at 550 nm using a spectrophotometer. The residual cholesterol concentration in the CFS was calculated according to Rudel & Morris [50]. The capability of the bacterium to assimilate cholesterol was then calculated using the following equation:

$$\text{Cholesterol assimilation (\%)} = [(\text{Cholesterol (}\mu\text{g/mL)}_{0\,\text{h}} - \text{Cholesterol (}\mu\text{g/mL)}_{72\,\text{h}})/\text{Cholesterol (}\mu\text{g/mL)}_{0\,\text{h}}] \times 100 \quad (1)$$

where, $(\text{Cholesterol})_{0\text{h}}$ represents the residual cholesterol concentration in CFS at 0-h of incubation, and $(\text{Cholesterol})_{72\,\text{h}}$ represents the residual cholesterol concentration in CFS at 72-h of incubation.

2.10. Safety Evaluation of LpBCC4352

2.10.1. Hemolytic Activity Assay

The hemolytic activity of *Lpb*BCC4352 was assessed using a plate assay following the procedure outlined by Islam et al. [24] with slight modifications. A one-loopful of the overnight culture of *Lpb*BCC4352 was streaked onto 0.5% (*v*/*v*) sheep blood agar plate and then incubated at 37 °C for 48 h. The plate was subsequently inspected for the development of a distinct translucent zone surrounding the culture-streaked line, serving as an indicator of a hemolytic reaction. *Staphylococcus aureus* ATCC 6538 was employed as a beta-hemolytic (positive) control [51], while *L. plantarum* NCIMB 8826 served as the hemolytic negative control (gamma-hemolytic) [52].

2.10.2. Antibiotic Susceptibility Test

The antibiotic susceptibility profile of *Lpb*BCC4352 against a range of antibiotics, including ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, and tetracycline, was determined through the micro-dilution method [53]. Colonies of *Lpb*BCC4352 grown anaerobically on MRS agar at 37 °C for 16–24 h were carefully selected and suspended in 5 mL of sterile 0.85% saline solution to make a cell suspension with an optical density (OD_{625nm}) of 0.16–0.2. Subsequently, the bacterial suspension underwent a 500-fold dilution in the susceptibility test medium, composed of 90% Iso-Sensitest (IST) medium and 10% MRS medium, before being distributed into each well of a 96-well plate that contained varying concentrations of antibiotics. The minimum inhibitory concentration (MIC) values were then visually assessed after 48 h of anaerobic incubation at 37 °C.

2.11. Evaluating the Performance of LpbBCC4352 as a Starter Culture in Nham Production

2.11.1. Starter Culture Preparation

*Lpb*BCC4352 cells cultured overnight in MRS broth were harvested by centrifugation at 8500× *g* for 10 min at 4 °C. Subsequently, the harvested cells were washed twice with a saline solution (0.85% NaCl) and then resuspended in the same diluent to make a final cell concentration of 9 log CFU/mL.

2.11.2. Nham Preparation

Nham was prepared in a private meat processing plant located in Bangkok, Thailand, following the formulation detailed by Visessanguan et al. [20]. In summary, two different batches of Nham were prepared with *Lpb*BCC4352 inoculation: one with an initial count of 4 log CFU/g (Nham-Lp4) and the other with 6 log CFU/g (Nham-Lp6). A control group, which consisted of un-inoculated naturally fermented Nham, was labeled as Nham-NF. The fermentation process for all Nham samples took place at a temperature of 30 °C for 96 h. Samples of Nham were collected and analyzed every 12 h throughout the fermentation period.

2.11.3. Determination of Physicochemical Properties of Nham

At each sampling point, Nham samples were analyzed for titratable acidity (TA), pH, texture, and color. Titratable acidity (TA) was determined following the AOAC method [54] and expressed as grams of lactic acid per 100 g of Nham (on a dry weight basis). The pH was measured using a standard pH meter (Mettler Toledo, Greifensee, Switzerland). The texture profile analysis (TPA) of Nham was conducted using a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a cylindrical aluminum probe (50 mm diameter) as detailed by Visessanguan et al. [20]. Color was evaluated in the CIE *L*a*b** color space using a Minolta Colour Meter CR300 (Minolta Camera Ltd., Osaka, Japan). To address color variations, three readings were averaged from the surface of each sample as described by Visessanguan et al. [20]. Total color difference between control sample (Nham-NF) and treatments (Nham-Lp4 and Nham-Lp6) was calculated according to Sharma [55].

2.11.4. Microbiological Analysis of Nham

The total LAB count in Nham was determined using the standard plate count technique. Triplicate samples of Nham (25 g each) were mixed with 225 mL of sterilized 1% peptone solution and homogenized for 1 min at 200 rpm using a stomacher (Seward, West Sussex, UK). Ten-fold serial dilutions were prepared, and the dilutions were plated on MRS agar plates supplemented with 0.75% (*w*/*v*) $CaCO_3$. The plates were then incubated at 30 °C for 48 h before enumerating the colonies.

2.11.5. Sensory Analysis

The sensory analysis of Nham was conducted following the procedure outlined in our previous study [21]. Briefly, a group of 30 individuals, regular consumers of Nham, were tasked with assessing the sensory characteristics of Nham. Before the evaluation, Nham samples with a pH of not more than 4.6 were left to reach room temperature for a minimum of 30 min and then randomly distributed to the panelists. Each sensory aspect was assessed on a hedonic scale ranging from 1 to 9, where panelists indicated their score based on their perception, with 1 signifying extreme dislike, 5 indicating neither liking nor disliking, and 9 representing extreme liking.

2.12. Statistical Analysis

Statistical data differentiation was conducted through one-way analysis of variance (ANOVA). Significance in mean differences among data groups was determined when p-value ≤ 0.05. Duncan's multiple range test was employed to assess these mean differences.

3. Results and Discussion

3.1. Antimicrobial Activity

Lactobacilli are recognized for their ability to produce diverse antimicrobial compounds that effectively inhibit the growth of pathogenic bacteria, thereby contributing significantly to their probiotic activity [56]. *Lpb*BCC4352 exhibited antimicrobial activity against *K. rhizophila* NBRC 12708^T, *L. plantarum* ATCC 14917^T, *L. sakei* JCM 1157^T, *P. pentosaceus* JCM 5885^T, and the zoonotic *S. suis* strains P1/7 through the cell-to-cells spot-on-lawn assay. Nonetheless, its neutralized cell-free supernatant did not show antimicrobial activity against any of the tested bacterial indicators (Table 1).

These results suggest that antimicrobial production by *Lpb*BCC4352 requires co-cultivation with specific bacterial strains. Antimicrobial production of lactobacilli through co-culture fermentation has been widely reported [57–61]. Through the investigation, *S. suis* is recognized for its high contagiousness, with the potential to cause permanent hearing impairment and fatality in humans [62–65]. Although documented outbreaks of *S. suis* are not prevalent in Thailand, concerns regarding consumer safety have arisen due to reported instances of *S. suis* contamination in raw pork meat, a major ingredient of Nham [65–67]. In this study, the observed capability of *Lpb*BCC4352 to inhibit *S. suis* sug-

gests a potential application of these bacteria to enhance the safety of Nham consumption. Ongoing research is expected to further investigate this prospect.

3.2. Resistance to pH, Bile Salts, and the Simulated Digestive Conditions

Evaluating the ability of LAB strains to withstand physical and chemical stress barriers in the gastrointestinal tract, such as gastric acidity and bile toxicity, is commonly recommended for identifying potential probiotic strains [68]. In this study, *Lpb*BCC4352 displayed remarkable acid tolerance, with a cell viability of 6.3 log CFU/mL after exposure to pH 2.5 for 2 h and achieving a survival rate exceeding 96.9 ± 0.1% (Figure 1a,b) while *Lpb*NCIMB 8826, a positive control, exhibited resilience to pH 2.5 with a cell viability of 6.6 log CFU/mL and a survival rate of 98.9 ± 0.1% (Figure 1a,b). The ability of Lactiplantibacillus to withstand low pH values is a significant aspect of pH homeostasis, a process in which H^+-ATPase, glutamate-decarboxylase, and arginine-deiminase play essential roles [69–72].

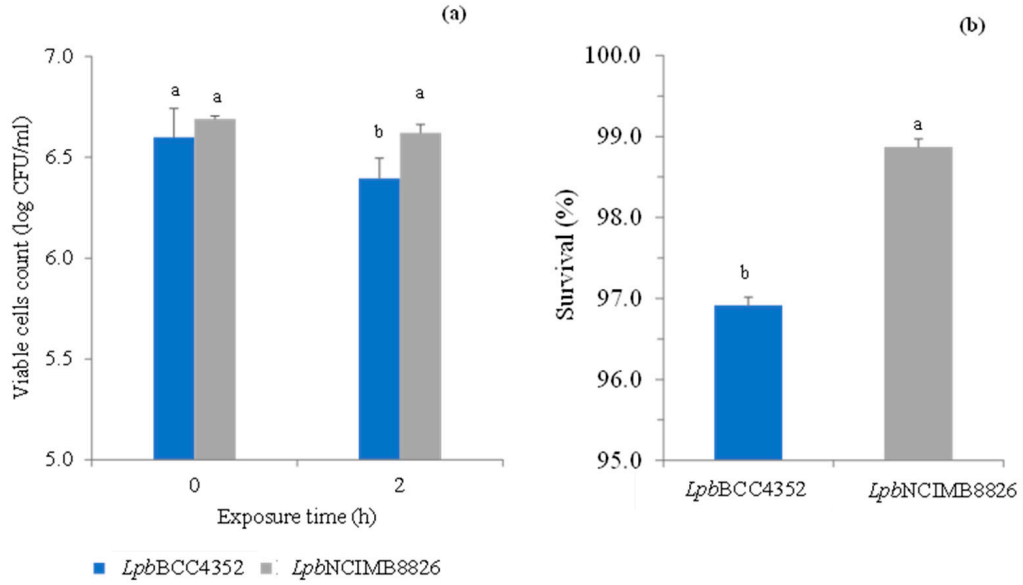

Figure 1. Acid tolerance test of *L. plantarum* strains. (**a**) viable cell count after acid exposure for 2 h, (**b**) % survivability after acid treatment. Data represent the means of triplicates. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different lowercase letters indicate significant differences among tested strains at the same exposure time ($p \leq 0.05$).

During the human digestive process, bile salts are initially released from the gallbladder, with concentrations ranging from 1.5% to 2.0% during the first hour of digestion and gradually decreasing to around 0.3% (*w*/*v*) or lower [73]. Therefore, a concentration range of 0.1% to 2.0% (*w*/*v*) of bile salts was employed in the test medium to assess the survival of tested strains. The results indicated that variations in oxgall concentration in the fermentation medium had a significant impact on the growth of tested strains. The viability of both *Lpb*BCC4352 and *Lpb*NCIMB8826 decreased as the concentrations of oxgall in the medium increased, particularly at concentrations higher than 0.3% (Figure 2a). After a 4-h exposure to the tested medium, *Lpb*BCC4352 demonstrated resistance against the tested bile salt. Even when exposed to the highest concentration of oxgall (2%), *Lpb*BCC4352 maintained a noteworthy viability of 75.2 ± 2.8% (Figure 2b) and a cell viability of 7.2 log CFU/mL (Figure 2a). In contrast, *Lpb*NCIMB8826 exhibited a survivability of 79.4 ± 0.7% (Figure 2b) and displayed a cell viability count of 7.9 log CFU/mL (Figure 2a). These findings suggest that *Lpb*BCC4352 exhibits notable in vitro bile tolerance when compared to the probiotic reference strain.

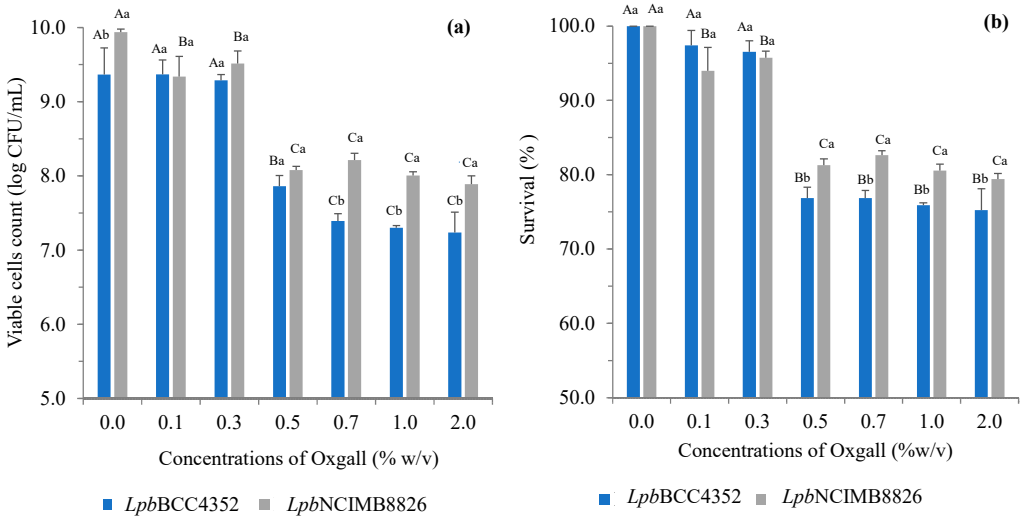

Figure 2. The effects of bile salts concentrations on growth of *L. plantarum* strains. The strains were exposed to the tested bile at 37 °C for 4 h. (**a**) the viable cell counts after bile salt exposure for 4 h and (**b**) the % survivability after bile salt treatments are shown. Data represent the means of triplicates. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Uppercase letters represent significant differences among the oxgall concentrations of the same strain ($p \leq 0.05$). Lowercase letters represent significant differences between the strains at the same oxgall concentration ($p \leq 0.05$).

Contrary to expectations, our findings revealed that *Lpb*BCC4352 lacked in bile salt hydrolase (BSH) activity in various test media, such as MRS-TDCA and MRS-GDCA (Figure S1). This was surprising given the commonly established correlation between bile tolerance in lactobacilli and BSH activity. This implies that the bile tolerance observed in *Lpb*BCC4352 may be linked to metabolic mechanisms beyond BSH activity, possibly related to the roles of proteins that impact cell envelope architecture or uphold intracellular homeostasis [74–77].

The survivability of *Lpb*BCC4352 cells in a simulated human digestive model is illustrated in Figure 3. When exposed to SSF containing amylase, *Lpb*BCC4352 demonstrated a viability exceeding 90%, with a retained cell count of 8.6 log CFU/mL. However, sequential exposure to SGF for 2 h resulted in a further slight reduction in the survival count by 1.0 log cycles, with the count retaining 7.6 log CFU/mL. After an additional 4 h of exposure to SIF, viability decreased further by an average of 1.2 log cycles, and the final cell count reached 6.4 log CFU/mL. Similar results were obtained when *Lpb*NCIMB8826 was subjected to the tested model; a decline in cell count was observed when the strain was sequentially exposed to SIF. The cell viability is retained at 6.9 log CFU/mL at this stage. The obtained results are aligned with those from experiments on acid and bile tolerance. These results suggest that *Lpb*BCC4352 has the potential to survive passage through the digestive tract.

3.3. Adhesion to Caco-2 Cells and Mucin

The ability of strains to adhere to intestinal epithelial cells and mucosal surfaces, crucial in conferring health benefits by competing with pathogens to colonize epithelial cells and modulating the host immune system, is considered as a primary criterion in the selection of probiotic microorganisms [78–80]. Figure 4a reveals a significantly higher adhesion level of *Lpb*BCC4352 when compared with *Lpb*NCIMB8826 ($p < 0.05$). *Lpb*BCC4352 cells demonstrated an adhesion rate of 84.5 ± 0.1%, while *Lpb*NCIMB8826 exhibited a lower rate of 77.7 ± 0.1%. However, *Lpb*BCC4352 exhibited lower mucin adherence (74.8 ± 1.1%) than *Lpb*NCIMB8826 (79.9 ± 1.9%), as shown in Figure 4b.

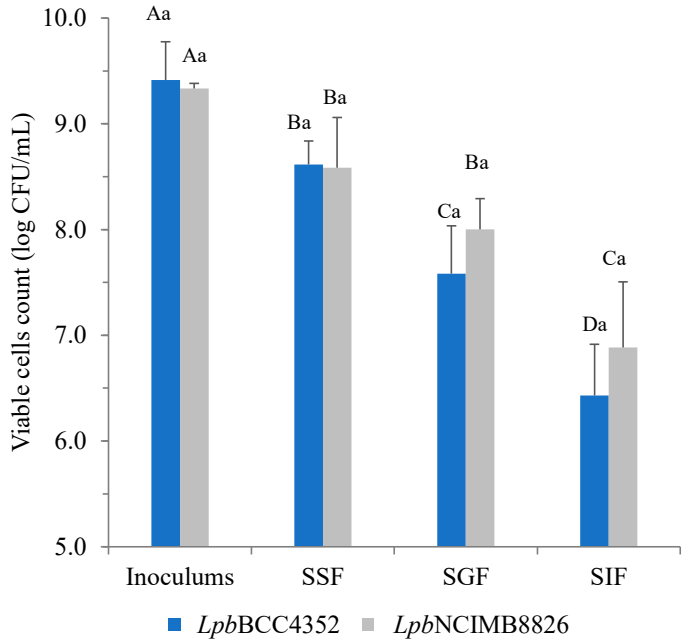

Figure 3. Survival of *L. plantarum* strains during transit to the simulated conditions of the human digestive tract. Data represent the means of triplicates. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different uppercase letters indicate significant differences among the stages of the same strain ($p \leq 0.05$). Different lowercase letters indicate significant differences between the tested strains at the same stage ($p \leq 0.05$).

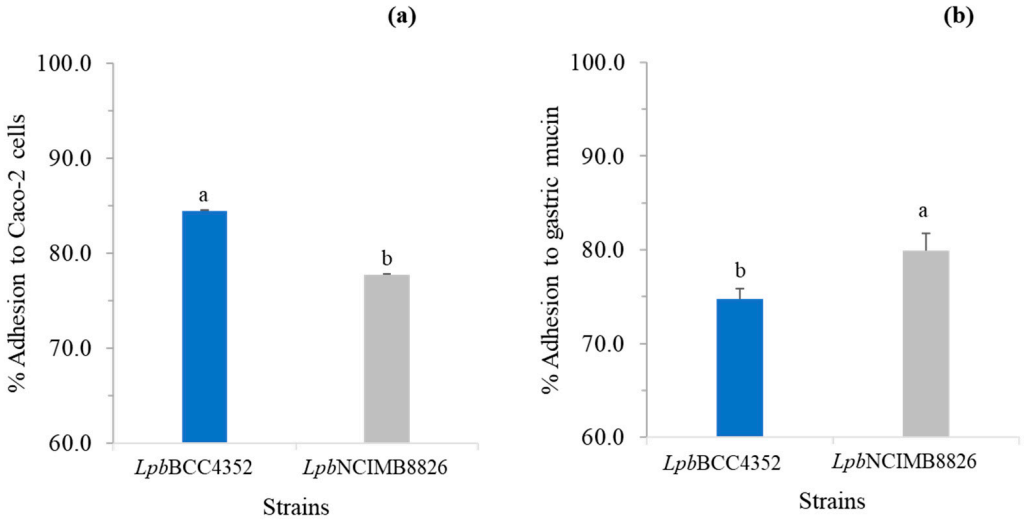

Figure 4. Bacterial adherence to Caco-2 cells (**a**) and gastric mucin (**b**). The capability of bacteria to adhere to Caco-2 cells and mucin was expressed as a percentage of adhesion. Data represent the means of triplicates. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different lowercase letters indicate significant differences between the tested strains in a specific adhesion test ($p \leq 0.05$).

We investigated the presence or absence of genes related to the binding ability of lactobacilli to the gastrointestinal tract, as proposed by Turpin et al. [45]. The analysis revealed the presence of binding-related genes in *Lpb*BCC4352, including aggregation-promoting factors (*apf*), collagen-binding protein (*cnb*), fibronectin-binding protein (*fpbA*), mucus adhesion promoting protein (*mapA*), glucan synthase (*gtf*), mannose-specific adhesion (*msa*), mucin-binding protein (*mub1, mub2*), and surface layer protein A (*slpA*) as shown in Table 2. The agarose gel electrophoresis of PCR products is presented in Figure S2.

Table 2. The distribution of genes involved in binding to the gastrointestinal tract in the selected *L. plantarum*.

Strains	Binding-Related Genes										References
	apf	***cbsA***	***cnb***	***fpbA***	***mapA***	***gtf***	***msa***	***mub1***	***mub2***	***slpA***	
L. plantarum BCC4352 (*Lpb*BCC4352)	+	-	+	+	+	+	+	-	+	+	This study
L. plantarum NCIMB 8826 (*Lpb*NCIMB8826)	+	+	+	+	+	+	+	+	+	-	This study
L. plantarum 11.3	+	-	+	+	+	-	+	+	+	-	[45]
L. plantarum 2.1	+	-	-	+	+	-	-	+	+	-	[45]
L. plantarum A6	+	-	+	+	+	-	+	+	+	-	[45]

Regarding adherence to Caco-2 cells, *Lpb*BCC4352 exhibits a significantly higher adhesion rate compared to *Lpb*NCIMB8826. This is possibly due to the presence of the *SlpA*, which is absent in *Lpb*NCIMB8826. *SlpA* was reported to be involved in the attachment of bacterium to Caco-2. *SlpA* from *L. acidophilus* NCFM was shown to engage in binding with both Caco-2 cells and dendritic cells (DC), influencing the modulation of DC and T-cell functions, as demonstrated in studies by Buck et al. [81] and Ashida et al. [82].

Furthermore, the obtained results indicate a potential involvement of mucin-binding proteins (*mub1*, *mub2*) and the collagen-binding S-layer (*cbsA*) in enhancing the binding capabilities of the tested strains to mucin. In particular, the adhesive strength of *Lpb*NCIMB8826 seems to be enhanced by the presence of *mub1*, *mub2*, and *cbsA*. The significant difference in the binding capacity of *Lpb*NCIMB8826 and *Lpb*BCC4352 emphasizes the impact of the absence of *mub1* and *cbsA* in *Lpb*BCC4352. Nevertheless, further study of the expression of the corresponding genes (*SlpA*, *mub1*, *mub2*, and *cbsA*) is needed to confirm their involvement in the binding mechanism in *Lpb*BCC4352.

3.4. Growth in the Prebiotic Medium

The results demonstrated that *Lpb*BCC4352 could utilize FOS in the medium tested, as evidenced by observed cell growth, pH reduction, and acid production. *Lpb*BCC4352 displayed robust growth behavior, manifesting a significant rise in cell counts from the initial 5.2 to 7.2 log CFU/mL within 24 h of incubation, followed by a slight increase to 7.5 log CFU/mL at 48 h of incubation (Figure 5a). Besides the observed growth, the decline in pH value from 6.7 to 4.3 during the growth phase (Figure 5b) and the concurrent rise in the production of acids at 48 h (Figure 6) of fermentation consistently support the fermentability of the fructo-oligosaccharides by *Lpb*BCC4352.

Similar results were observed when growing *Lpb*BCC4352 in mSIEM medium containing 2% glucose. There was a rise in *Lpb*BCC4352 cell counts from the initial values of 5.2 to 7.3 log CFU/mL within 24 h of incubation, although a slight decrease of 0.46 log cycles was observed at 48-h of fermentation (Figure 5a). The rapid decline in pH value from 6.6 to 3.7 during the growth phase was noted (Figure 5b). The production of targeted SCFAs was found to be similar to that detected in the mSIEM medium with 2% FOS. However, a lower amount of valeric acid was investigated (Figure 6).

Compared to the basal mSIEM medium, *Lpb*BCC4352 cell viability consistently remained at 5 log CFU/mL throughout the fermentation period (Figure 5a). The quantity of the targeted SCFAs produced was determined to be considerably lower than those observed in the mSIEM medium containing FOS and glucose (Figure 6).

*Lpb*BCC4352 was found to generate notably high concentrations of targeted short-chain fatty acids (SCFAs), with a particular emphasis on valeric acid production. *Lpb*BCC4352 exhibited outstanding performance, achieving a production level of 15.8 $\pm$ 1.25 mg/mL within a 48-h growth period. Valeric acid is recognized as a potential therapeutic target for various disease pathologies, such as cancer and colitis [83].

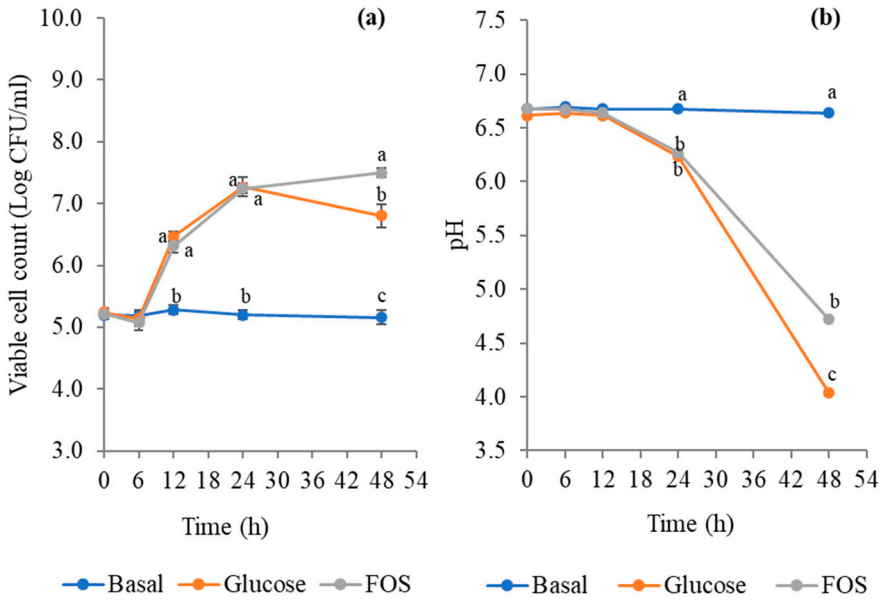

Figure 5. Changes in the growth of *L. plantarum* BCC4352 (**a**) and pH (**b**) during growth in mSIEM medium with and without the addition of tested carbohydrates. The strain was grown at 37 °C for 48 h. Values represent the means ± S.D. of triplicate determinations. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different lowercase letters indicate significant differences among the culture media at the same fermentation time ($p \leq 0.05$).

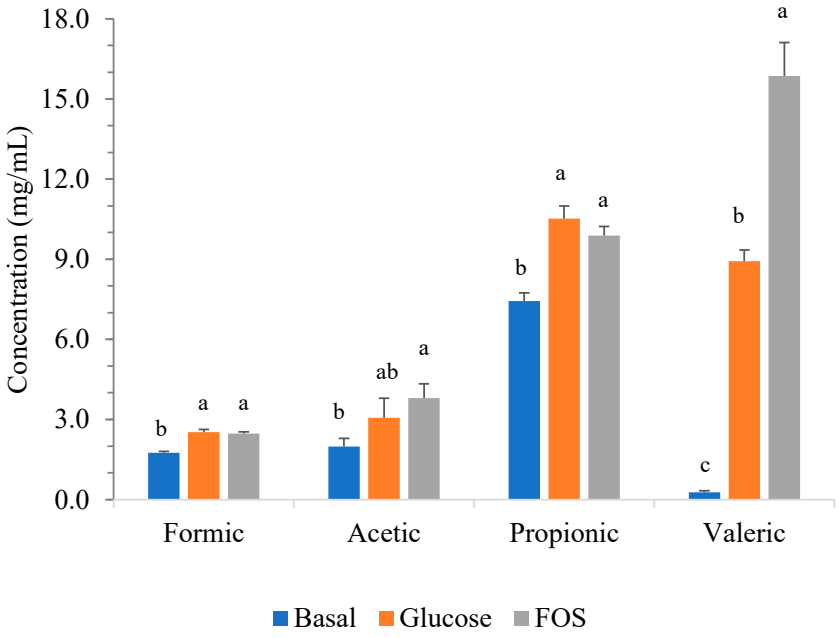

Figure 6. Short-chain fatty acid (SCFA) production during the growth of *Lpb*BCC4352 in mSIEM medium with and without the addition of tested carbohydrates. The strain was grown at 37 °C for 48 h, and all measurements were performed in triplicate. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different lowercase letters indicate significant differences among the tested carbohydrates of the same SCFA ($p \leq 0.05$).

3.5. Cholesterol Assimilation

Cholesterol assimilation refers to the mechanisms involved in the removal of cholesterol, which represents a desirable characteristic of probiotics [84]. The findings indicated that *Lpb*BCC4352 displayed a cholesterol assimilation of 42.27 ± 1.69%, resulting in the removal of 9.09 ± 0.80 μg/mL of cholesterol. In comparison, *Lpb*NCIMB8826 demon-

strated a higher cholesterol assimilation of 55.85 ± 7.80%, leading to the removal of 12.20 ± 1.21 µg/mL of cholesterol compared to the control medium without bacterial inoculation (Table 3). Our findings align with earlier studies [49,85,86], which documented the capability of lactobacilli to assimilate cholesterol from the fermentation medium. Lactobacilli are believed to remove cholesterol by integrating it into their cellular membrane, as suggested by various studies [49,85,87–90]. Further to these, alternative or additional mechanisms for cholesterol removal have been reported, including enzymatic deconjugation of bile acids, co-precipitation of cholesterol with deconjugated bile, cholesterol binding to cell walls [49,91,92], conversion of cholesterol into coprostanol [89], and the production of short-chain fatty acids by probiotics [93].

Table 3. Cholesterol reduction capability of *L. plantarum* strains.

Samples	Residual Cholesterol (µg/mL)		Cholesterol Reduction (µg/mL)	Cholesterol Assimilation (%)
	0 h	72 h		
*Lpb*NCIMB8826	20.70 ± 0.92 [a]	9.14 ± 1.21 [a]	12.20 ± 1.21 [a]	55.85 ± 7.80 [a]
*Lpb*BCC4352	20.16 ± 0.80 [a]	11.64 ± 0.80 [b]	9.09 ± 0.80 [b]	42.27 ± 1.69 [b]

Data represents the mean ± SD of triplicate determinations. Different small letters in the same column indicate significant differences ($p \leq 0.05$).

3.6. Hemolytic Activity

The hemolytic activity assay demonstrated that *Lpb*BCC4352 displayed a gamma-hemolytic property (non-hemolytic) on sheep blood agar, even after 48 h of incubation (Figure 7). Our findings align with previous studies by Osmanagaoglu et al. [94] and Gao et al. [95], supporting the notion that hemolysis is not a prevalent feature in lactobacilli. This observation underscores that *Lpb*BCC4352 satisfies the essential safety criteria for probiotics, as outlined in the FAO/WHO guidelines [96].

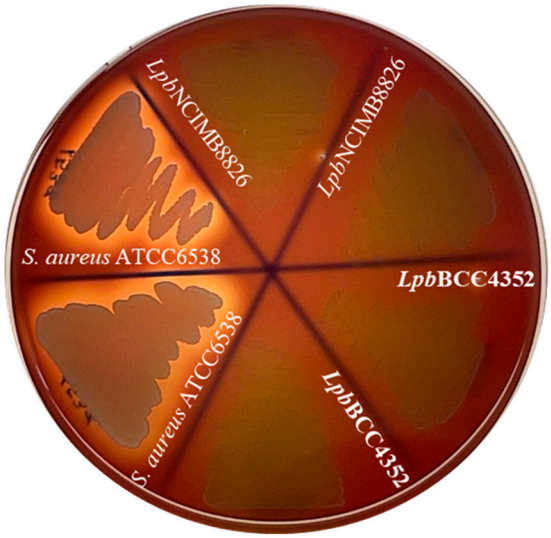

Figure 7. Hemolytic activity assay on sheep blood agar plates. Plates were incubated at 37 °C for 48 h before inspecting for hemolysis. The translucent zone surrounding the culture-streaked line indicates a positive beta-hemolytic reaction. *Staphylococcus aureus* ATCC6538 was employed as a beta-hemolytic (positive) control, while *L. plantarum* NCIMB8826 used as the gamma-hemolytic (negative) control.

3.7. Antibiotics Susceptibility Test

According to the Qualified Presumption of Safety (QPS) approach established by the European Food Safety Authority [97], it is imperative to assess the antibiotic resistance determinant in a probiotic candidate. The results revealed that *Lpb*BCC4352 exhibited sensitivity to all tested antibiotics listed in Table 4. These findings indicate that *Lpb*BCC4352

may be considered a safe strain for use as a probiotic or food starter culture, given its lack of antibiotic resistance to commonly used antibiotics.

Table 4. Antibiotics susceptibility profile of *L. plantarum* BCC4352 (*Lpb*BCC4352).

Antimicrobials	Drug Class	Test Range (mg/L)	MIC (mg/L)		Interpretation
			Cut Off [97]	*Lpb*BCC4352	
Ampicillin	Beta-lactam	0.06–8	2	0.5	Susceptible
Chloramphenicol	Phenicol	0.25–32	8	8	Susceptible
Clindamycin	Lincomycin	0.13–16	4	<0.13	Susceptible
Erythromycin	Macrolide	0.13–16	1	1	Susceptible
Gentamicin	Aminoglycoside	0.5–64	16	8	Susceptible
Kanamycin	Aminoglycoside	4–512	64	64	Susceptible
Tetracycline	Tetracycline	1–128	32	16	Susceptible

Values represent the means of triplicate determinations.

3.8. Effect of LpbBCC4352 on the Fermentation Characteristics of Nham

Physicochemical characterizations were employed to evaluate the fermentation characteristics of Nham, both in its natural fermentation product (Nham-NF) and when inoculated with *Lpb*BCC4352 (Nham-Lp4 and Nham-Lp6). Nham-Lp6 completed fermentation (pH $\leq$ 4.6) within 36 h, whereas both Nham-Lp4 and Nham-NF completed fermentation within 48 h (Figure 8a). These findings suggest that the pH development in Nham is influenced by the initial number of starter cultures added. Similar results regarding pH development in Nham, fermented with *L. curvatus* at initial inoculations of 4 and 6 log CFU/g, were reported by Visessanguan et al. [20]. An increase in titratable acidity was noted throughout the fermentation period (Figure 8b). The results indicate that the progression of titratable acidity in Nham-Lp6 surpassed that in both Nham-Lp4 and Nham-NF during the entire fermentation process.

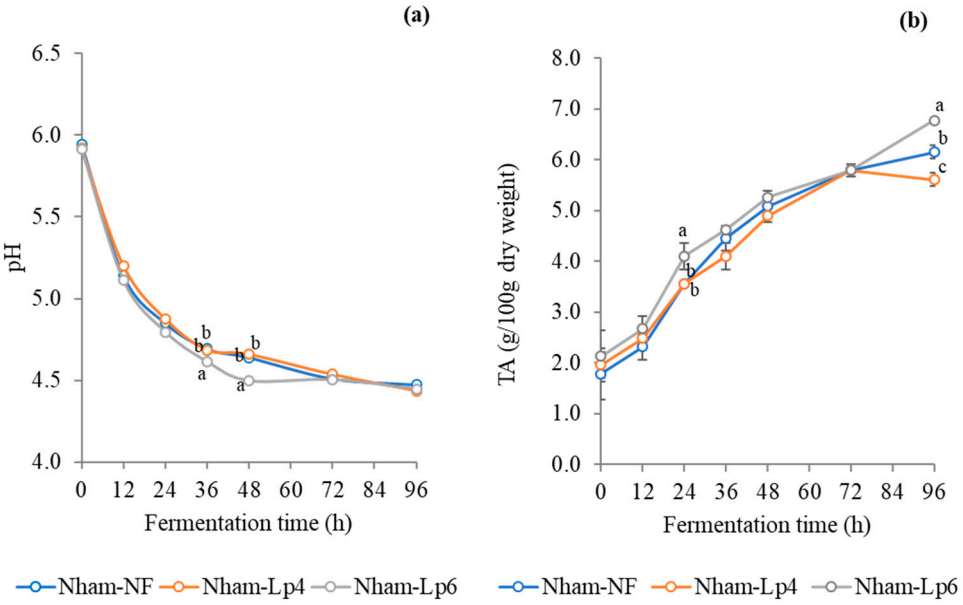

Figure 8. Changes in pH (**a**) and titratable acidity (**b**) of Nham, both with and without inoculation of *Lpb*BCC4352, were monitored during fermentation at 30 °C. The measurements were performed in triplicate. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different lowercase letters indicate significant differences ($p \leq 0.05$) among sample at the same fermentation time.

Inoculation with 6 log CFU/g of *Lpb*BCC4352 demonstrated the potential to enhance the firmness of Nham, as evidenced by the higher hardness value observed at 72–96 h of fermentation, compared to Nham-Lp4 and Nham-NF (Figure 9).

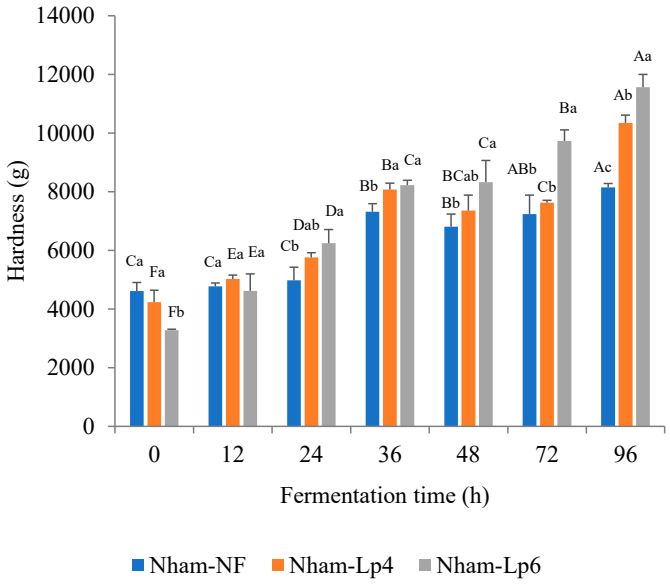

Figure 9. Changes in hardness of Nham, with and without inoculation of *Lpb*BCC4352, were monitored during fermentation at 30 °C. The measurements were performed in triplicates. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different uppercase letters indicate significant differences between fermentation times of the same sample ($p \leq 0.05$). Different lowercase letters indicate significant differences between samples at the same fermentation time ($p \leq 0.05$).

No significant differences in the color of Nham were detected among different levels of the *Lpb*BCC4352 starter culture ($p > 0.05$) (Table S2). At the end of the fermentation period, the total color differences (ΔE) values of Nham-Lp4 and Nham-Lp6 compared with Nham-NF were below 2.3, indicating that there were no noticeable differences in color between the samples that could be visualized by the naked eye [55]. Similar results regarding color development in Nham inoculated with *Lactobacillus curvatus* have been previously documented [20,37].

The numbers of LAB detected in the *Lpb*BCC4352-inoculated Nham increased drastically to a maximum of 10 log CFU/g within 24 h of fermentation and remained stable until 96 h of fermentation. In Nham-NF, there was a rise in the number of LAB to a maximum of 10 log CFU/g observed at 24 h of fermentation, and it tended to decrease after 48 h throughout the fermentation period. During 72–96 h of fermentation, LAB count in Nham-NF was significantly lower than those of Nham-Lp4 and Nham-Lp6 ($p < 0.05$) (Figure 10).

3.9. Acceptability Testing of the Finished Products

The results presented in Figure 11 indicate that there were no significant differences ($p > 0.05$) in consumer acceptability for the overall characteristics among three groups of Nham. The findings suggest that inoculating *Lpb*BCC4352 at various levels did not negatively impact the organoleptic properties of Nham. Consequently, *Lpb*BCC4352 could be used as a starter culture for Nham fermentation without compromising the distinctive quality of the product.

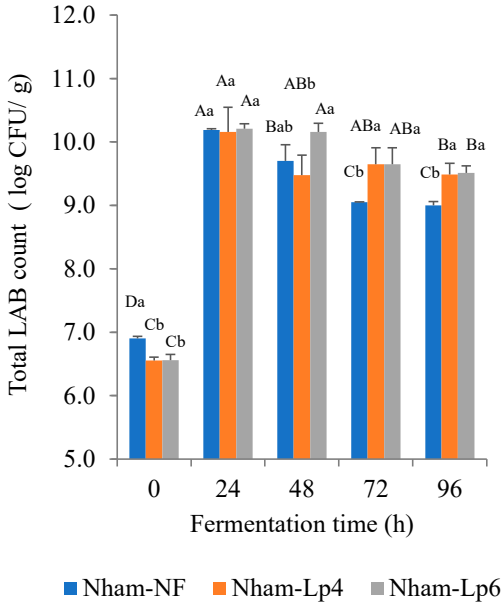

Figure 10. Changes in LAB count during fermentation of Nham at 37 °C for 96 h. The measurements were performed in triplicate. Significant differences were analyzed by one-way ANOVA with Duncan's multiple range test. Different uppercase letters indicate significant differences between fermentation times of the same sample ($p \leq 0.05$). Different lowercase letters indicate significant differences between samples at the same fermentation time ($p \leq 0.05$).

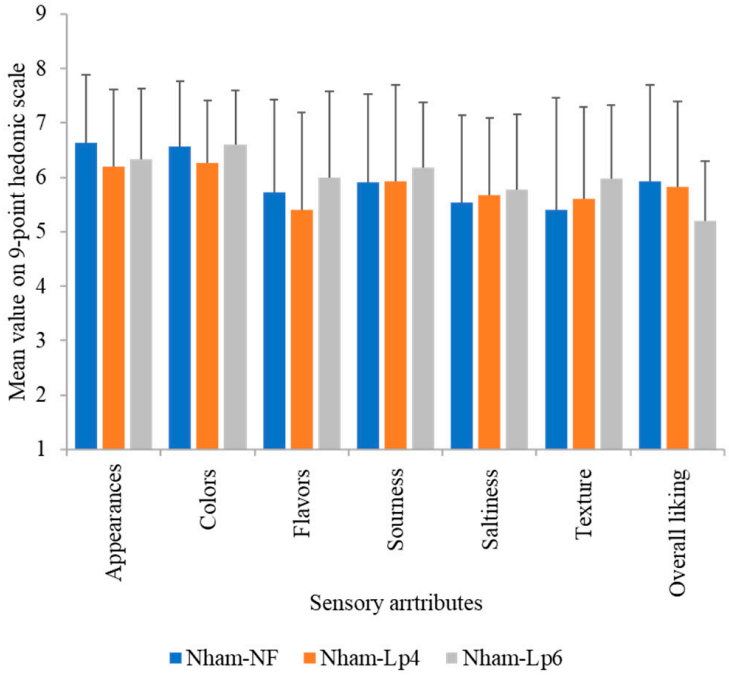

Figure 11. Sensory analysis of the final fermented Nham (pH < 4.6) with and without inoculation of *Lpb*BCC4352. Data are means of consumer judgements on a nine-point hedonic scale (n = 30).

4. Conclusions

L. plantarum BCC4352 exhibited desirable in vitro probiotic properties. Its ability to survive during transit through the simulated conditions of the human digestive process, along with a capability to adhere to epithelial cells and gastric mucin, indicates the possibility of its ability to proliferate and colonize in the gut. In addition, the ability to produce antimicrobials, utilize prebiotic carbohydrates, and remove cholesterol would be beneficial for its probiotic functions. In a food model, *Lpb*BCC4352 does not significantly affect the

unique character of the final fermented Nham when applied as a starter culture, indicating the potential application of this strain as a starter for Nham fermentation. Furthermore, Nham possesses the capability to act as a means of delivering a potentially probiotic strain *Lpb*BCC4352 to consumers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10030145/s1, Figure S1: Bile salts hydrolase (Bsh) activity assay of *L. plantarum* BCC4352; Figure S2: Agarose gel electrophoresis of PCR products from *L. plantarum* BCC4352 (a), *L. plantarum* NCIMB8826 (b), and negative control (c), respectively; Table S1. Primers used to detect the potential binding related genes in L. plantarum strains; Table S2. Changes in color of Nham before (t = 0 h) and after (t = 96 h) fermentation.

Author Contributions: Conceptualization, Y.K., A.P. (Atikorn Panya), A.P. (Awanwee Petchkongkaew) and W.W.; Methodology, Y.K., L.P., S.A., K.C., P.S., M.K., T.J., K.K., N.P., A.P. (Atikorn Panya), W.V., A.P. (Awanwee Petchkongkaew) and W.W.; Validation, Y.K., A.P. (Atikorn Panya), W.V., A.P. (Awanwee Petchkongkaew) and W.W.; Formal Analysis, Y.K., A.P. (Atikorn Panya), A.P. (Awanwee Petchkongkaew) and W.W.; Investigation, Y.K., L.P., S.A., K.C., P.S., M.K., T.J., K.K., N.P., A.P. (Atikorn Panya), W.V., A.P. (Awanwee Petchkongkaew) and W.W.; Resources, Y.K., A.P. (Atikorn Panya), W.V., A.P. (Awanwee Petchkongkaew) and W.W.; Writing—Original Draft Preparation, Y.K. and W.W.; Writing—Review & Editing, Y.K., W.V., A.P. (Atikorn Panya), A.P. (Awanwee Petchkongkaew) and W.W.; Supervision, W.V. and A.P. (Awanwee Petchkongkaew); Project Administration, Y.K. and W.W.; Funding Acquisition, W.V., A.P. (Awanwee Petchkongkaew) and W.W. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for sensory evaluation experiment because the study was performed before the Institutional Review Board was established.

Informed Consent Statement: Inform consent was obtained from all subjects involved in the sensory evaluation study.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors declare no conflict of interest.

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