Online Version: http://journal.ipb.ac.id/index.php/jtip
https://doi.org/10.6066/jtip.2026.37.3.205
Research Article

Jurnal Teknologi dan Industri Pangan/Journal of Food Technology and Industry
Vol. 37(3): 205-216 Th. 2026 ISSN: 1979-7788
Terakreditasi Kemdiktisaintek: 295/C/C3/KPT/2026

Check for updates

Growth Performance and Antibacterial Activity of *Lacticaseibacillus paracasei* D4 in Tofu Whey-Based Media

Muhammad Ismaeel[1,2], Yoga Dwi Jatmiko[1]*, Irfan Mustafa[1]

[1] Department of Biology, Faculty of Science, Technology, and Mathematics, Universitas Brawijaya, Malang, Indonesia
[2] Master Program of Biology, Department of Biology, Faculty of Science, Technology, and Mathematics, Universitas Brawijaya, Malang, Indonesia

Received April 2nd 2026 / Revised June 17th 2026 / Accepted July 23rd 2026

ABSTRACT

The development of low-cost and halal-compliant culture media for probiotic production is an important concern in food biotechnology. The cultivation of probiotic lactic acid bacteria (LAB) commonly relies on de Man, Rogosa, and Sharpe (MRS) broth, which is relatively expensive and may have uncertain halal status. This study evaluated tofu whey-based media as a halal and low-cost alternative for the cultivation and antibacterial metabolite production of *Lacticaseibacillus paracasei* D4. Three tofu whey-based media (Media A, B, and C), supplemented with different concentrations of molasses and skim milk, were compared with MRS broth. Antibacterial activity of non-neutralized and pH-neutralized cell-free supernatants was tested against *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium TRD1, and *Escherichia coli* ATCC 2TIN5922. During fermentation, pH decreased in all media, with Media C reaching the lowest final pH (2.84) and the highest bacterial population after five days (12.75±0.06 log CFU/mL), followed by MRS broth (11.71±0.03 log CFU/mL), Media B (11.69±0.04 log CFU/mL) and Media A (11.65±0.34 log CFU/mL). Medium C showed the strongest inhibition, with largest zones observed against *Staphylococcus aureus* ATCC 29213 (19.5 mm) and *Bacillus cereus* ATCC 11778 (14.5 mm). Carbohydrate content decreased during fermentation from 0.96–0.99% at day 0 to 0.52–0.69% at day 5. LC-HRMS analysis identified antimicrobial-associated metabolites, including diketopiperazines and cyclic dipeptides such as cyclo(Ile-Ser), cyclo(Pro-Val-Val), and Indole-3-lactic acid. These findings indicate that tofu whey-based media, particularly Media C, provide a promising halal and cost-effective alternative for probiotic cultivation and antimicrobial metabolite production.

Keywords: antibacterial activity, halal culture medium, *Lacticaseibacillus paracasei*, probiotic cultivation, tofu whey

INTRODUCTION

Lactic acid bacteria (LAB) are widely recognized as Gram-positive, non-spore-forming microbes that play a key role in fermenting carbohydrates into lactic acid (Alvarez-Sieiro *et al*., 2016). LAB are commonly used in the fermentation of foods such as dairy products, pickled vegetables, and fermented meats, where they play a role in developing flavor, improving texture, and maintaining product quality (Yang *et al*., 2024). The production of lactic acid enhances food safety by suppressing the growth of pathogenic microorganisms while also influencing flavor, aroma, and texture, thereby improving the overall sensory quality of food (Wang and Wang, 2014). *Lacticaseiplantibacillus paracasei* is considered one of the most extensively studied species of LAB due to its broad ecological distribution and metabolic flexibility (Syahniar *et al*., 2026). They are usually cultivated in de Man, Rogosa, and Sharpe (MRS) medium which contains vital nutrient including peptone, beef extract, yeast extract, dextrose, and selective salts that help LAB growth and inhibits other undesired microbes (Miranda and Nader-Macías, 2023). The use of MRS medium becomes challenging at an industrial level, mainly because it is expensive and may include animal-derived ingredients that raise halal concerns (Maulidiyah *et al*., 2024). As the production of safe, inexpensive, and halal-compliant probiotic products is becoming a growing demand, there is an urgent need to create alternative culture media that do not use non-halal or costly ingredients (Valle Vargas *et al*., 2024). The agro-industrial by-

*Corresponding Author: E-mail: jatmiko_yd@ub.ac.id

Copyright © 2026 by Authors, published by Journal of Food Technology and Industry
This is an open-access article distributed under the CC BY-SA 4.0 License
https://creativecommons.org/licenses/by-sa/4.0/

products, especially the tofu-whey, which is inexpensive, nutrient-dense, and naturally halal, have become a potential solution (Hadidi *et al*., 2024). Tofu whey may be used as a sustainable fermentation substrate that is able to support LAB growth without affecting probiotic activity (Tindjau *et al*., 2023a). In addition to promoting bacterial growth, LAB are known to produce antimicrobial metabolites, including organic acids, bacteriocins, and bioactive peptides, which can inhibit the growth of pathogenic microorganisms (Arena *et al.*, 2016a). *L. paracasei* has been reported to synthesize various antimicrobial compounds that contribute to its probiotic and biopreservative potential (Mutmainna *et al*., 2021). Therefore, evaluating both bacterial growth and antimicrobial activity is important to determine the suitability of alternative media for supporting the production of functional metabolites (Maulidiyah *et al*., 2024)

Indonesia has a reputation of various tofu production firms (Ginting *et al*., 2024). Whey is a liquid by-product of clumping, e.g. the manufacture of tofu and cheese (Kolesovs and Semjonovs, 2020). As a by-product of coagulation processes in tofu and cheese production, whey is commonly regarded as waste and, if not properly managed, can lead to environmental issues (Giulianetti de Almeida *et al*., 2023). Nevertheless, tofu whey is a promising source of nutrients to be used in the cultivation of LAB. Tofu whey includes organic compounds, such as crude protein, carbohydrates, ash, and fats (Wang *et al*., 2018). It is also a good source of carbon and nitrogen, and contains vitamins and minerals, which makes it an appropriate substrate to grow probiotics. Tofu whey media performance may be further improved with the addition of more nitrogen and carbon sources, including skim milk powder and molasses that increase the availability of proteins and the fermentable sugar content, respectively (Tindjau *et al*., 2023b). Agro-industrial by-products are good substitutes since they are highly available, not expensive, and contain high levels of nutrients (Kilama *et al*., 2023). Molasses, which is a by-product of sugar crystallization contains high sugar and minerals (Zhang *et al*., 2020). In addition to these agro-industrial by-products, skim milk was used as a supplementary nutrient source because it contains lactose, peptides, amino acids, vitamins, and minerals that facilitate the growth of LAB (Maselli and Hekmat, 2016).

Though many researchers have investigated the use of alternative media to grow LAB, most commercially available media still rely on expensive chemical compounds rather than food-related industrial by-products as nutrient sources (dos Santos *et al*., 2021). Moreover, antibacterial activities of LAB grown in newly halal media remains limited (Maulidiyah *et al*., 2024). Since this study relied solely on *in vitro* testing, it does not address how the probiotic would perform *in vivo* or how stable the medium remains over time, which restricts its real-world and industrial use. In this study, tofu whey was explored as a potential base for a low-cost, halal culture medium. The growth performance, physicochemical characteristics, and antibacterial activity of *L. paracasei* D4 were then evaluated and compared with those obtained using conventional MRS medium.

MATERIALS AND METHOD

Materials

The materials used in this study were the probiotic strain *L. paracasei* D4 (Microbiology Laboratory, Universitas Brawijaya, Malang, Indonesia), indicator or pathogenic bacteria (*Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, and *Salmonella* Typhimurium TRD1), tofu whey (SWT Tofu Factory, Malang, Indonesia), sugarcane molasses (Kedawoeng Sugarcane Factory, Pasuruan, Indonesia), skim milk powder (NZMP, New Zealand), de Man, Rogosa, and Sharpe (MRS) broth (Oxoid, UK), Mueller-Hinton Broth (MHB) (Himedia, India), Nutrient Broth (NB) (Oxoid), MRS agar (Oxoid), calcium carbonate ($CaCO_3$) (Merck, Germany), sodium hydroxide (NaOH) (Merck), hydrochloric acid (HCl) (Merck), saline water (NaCl 0.85%), chloramphenicol (30 ppm), sterile paper disks (6 mm, Whatman, UK), and distilled water.

Research design

An experimental laboratory study was conducted to examine the growth of *L. paracasei* D4 and the antimicrobial activity of its cell-free supernatant (CFS) derived from cultures grown in tofu whey-based medium. Three tofu whey-based media were tested in the experiment with the additions of skim milk and molasses versus a control in MRS broth. Fermentation was monitored over five days to evaluate changes in bacterial cell density, pH, proximate composition, and the antibacterial activity of the CFS.

Inoculum preparation

The probiotic strain *L. paracasei* D4 used in this study was obtained from the Microbiology Laboratory, Faculty of Science, Technology, and Mathematics, Universitas Brawijaya, Malang, East Java. To prepare the starter culture, 100 µL of glycerol stock (−80 °C) was revived in 30 mL of sterile MRS broth and incubated at 37 °C for 24 h. Following incubation, 1.5 mL of the culture was transferred into microtubes and washed three times with sterile saline solution (0.85% NaCl) by centrifugation at 4000 rpm for 5 min using

an Eppendorf 5424 R refrigerated microcentrifuge (Eppendorf, Germany) to remove residual MRS medium. For inoculum preparation, 30 mL of sterile MRS broth was inoculated with two loops of *L. paracasei* and incubated at 37 °C for 48 h. Gram staining confirmed that the cells were Gram-positive. A 1% (v/v) inoculum was used for fermentation. The culture was then serially diluted by transferring 10 µL of *L. paracasei* into 90 µL of sterile saline solution in microtube. The bacterial cell density was adjusted to 10^6 cells/mL using a hemocytometer and an Olympus CX23 optical microscope (Olympus, Japan) prior to use in fermentation.

Preparation of tofu whey-based media

Centrifugation of tofu whey was done at 4000 rpm at 4 °C for 5 min using an Eppendorf 5424 R refrigerated microcentrifuge to get the supernatant. Before sterilization, the pH of the tofu was adjusted to 6.0–6.5 using 2 M NaOH. Sugarcane molasses (30 g) was dissolved in 100 mL of distilled water to produce a solution with a concentration of 20 °Brix. Molasses was sterilized by autoclaving at 105 °C for 15 min. Skim milk powder used in this study was halal-certified (New Zealand Milk Products, NZMP). Skim milk powder (5 g) was dissolved in 100 mL of distilled water, and the resulting solution was pasteurized at 65 °C for 30 min before use. The components of the medium were sterilized separately. MRS and tofu whey were autoclaved at 121 °C (1 atm) for 15 minutes. After sterilization, the tofu whey, molasses, and skim milk solutions were aseptically combined in a laminar airflow cabinet according to the respective medium formulations prior to inoculation.

The medium formulations were medium A: tofu whey (90%), molasses (5%), and skim milk (5%); medium B: tofu whey (90%), molasses (2.5%), and skim milk (7.5%); medium C: tofu whey (90%), molasses (7.5%), and skim milk (2.5%); and medium MRS broth as a standard medium (medium D). These media were chosen on the basis of nutrient compatibility and past studies that prove the relevance of balanced carbohydrates and nitrogen media in LAB growth (Maulidiyah *et al*., 2024).

Fermentation and sampling schedule

The fermentation of *L. paracasei* D4 was performed for five days under static aerobic conditions at 37 °C. Fermentation was carried out in a total volume of 400 mL under controlled conditions. During fermentation, samples were obtained at four time points: day 0, 1, 3, and 5. Samples taken at these time points were used to assess bacterial growth, pH changes, and antibacterial activity during fermentation.

Bacterial density test

L. paracasei D4 was evaluated to determine the changes in bacterial density during the fermentation process. The viable cell count was determined using the standard plate count method (ISO, 2013). Serial dilutions (10^{-1} to 10^{-9}) were prepared using sterile saline solution under aseptic conditions, and 100 µL of each appropriate dilution was plated onto MRS agar supplemented with 1% $CaCO_3$. The plates were maintained at 37 °C for 48 h during incubation. After incubation, colonies with clear zones were observed and counted. The bacterial viability was then expressed as colony-forming units per milliliter (CFU/mL), calculated using Formula 1.

$$\frac{\text{CFU}}{\text{mL}} = \frac{(\text{Number of colonies} \times \text{Dilution factor})}{\text{Volume plated (mL)}} \quad \text{..... (1)}$$

Proximate analysis

Samples from the alternative media (Media A, B, and C) were analyzed for proximate composition on day 0 and day 5 of fermentation. For each sampling point, 40 mL of media was collected for analysis. Standard AOAC methods were used to determine moisture, ash, crude protein, crude fat, and carbohydrate contents (AOAC International, 2019). All proximate composition results were expressed on a wet weight basis (% w/w). Results from day 0 and day 5 were compared to evaluate changes in nutrient composition during fermentation and to assess nutrient utilization by *L. paracasei* D4.

Antibacterial activity assay

Samples for antibacterial testing were collected on day 1, 3, and 5 with 30 mL taken from each treatment. Two kinds of cell-free supernatants (CFS) were prepared from each sample: the original non-neutralized CFS and the neutralized CFS adjusted to pH 6.0–6.5. The original pH (non-neutralized) was maintained in the first supernatant to determine the natural antimicrobial effect generated during fermentation. The second supernatant was adjusted using 2 M NaOH to a pH 6.0–6.5 yielding the neutralized CFS. The pH adjustment reduced the antimicrobial effect of organic acids by shifting them into their dissociated form, enabling the remaining antibacterial activity to be attributed specifically to bacteriocin-like inhibitory substances (BLIS). Centrifugation was performed at 8,000 rpm for 10 min at 4 °C using an Eppendorf 5424 R refrigerated microcentrifuge following pH adjustment of the samples. The CFS obtained after centrifugation was collected, while the pellets were removed. The CFS was then freeze-dried, and 0.5 g of the freeze-dried supernatant was dissolved in 500 µL of sterile distilled water.

Four indicator bacteria or pathogenic bacteria namely *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 11778, *Salmonella* Typhimurium TRD1, and *Escherichia coli* ATCC 25922 were prepared. Bacterial stock (100 *μ*L) was added to 10 mL of sterile Nutrient Broth, and the culture was then incubated at 37 °C for 24 h. The bacterial suspension was then adjusted to an optical density (OD_{600}) of 0.5. The bacterial suspension was evenly swabbed onto Nutrient Agar. Chloramphenicol (30 ppm) was used as the positive control to validate the antibacterial activity assay. In the disk diffusion assay, 45 *μ*L of CFS sample was carefully applied onto sterile paper disks (6 mm in diameter). Disks were placed onto agar plates inoculated with the respective bacteria, followed by incubation at 37 °C for 24 h. The inhibition zones were then measured using Formula 2.

Inhibition zone (mm)=Dz-Dd ……(2)

where *Dz* shows total diameter of the inhibition zone (mm), and *Dd* shows diameter of the paper disk (mm) (6 mm)

Liquid chromatography–high resolution mass spectrometry (LC-HRMS) analysis

Cell-free supernatants were analyzed using liquid chromatography-high resolution mass spectrometry (LC-HRMS) (Thermo Scientific Q Exactive, USA) to identify antimicrobial and bacteriocin-related metabolites. Prior to analysis, 300 *μ*L of sample was diluted with a polar solvent to a final volume of 1500 *μ*L. The mixture was vortexed at 2000 rpm for 2 minutes and centrifuged at 6000 rpm for 2 minutes. The supernatant was collected, filtered through a 0.22 *μ*m syringe filter, and transferred into an amber vial for LC-HRMS injection. An injection volume of 5 *μ*L was used.

Chromatographic separation was performed using a Thermo Scientific Dionex Ultimate 3000 RSLCnano system equipped with a Hypersil GOLD column (50×1 mm, 1.9 *μ*m particle size), maintained at 30 °C. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, delivered at a flow rate of 40 *μ*L/min. The gradient was programmed from 5 to 95% B over 25 min, followed by re-equilibration to initial conditions. Mass spectrometric analysis was carried out using a Thermo Scientific Q Exactive high-resolution mass spectrometer operated in positive ionization mode. Full scan acquisition was performed over an *m/z* range of 100–1500 at a resolution of 70,000, followed by data-dependent MS/MS (dd-MS^2) acquisition at a resolution of 17,500. Data processing and compound identification were conducted using Compound Discoverer 3.3 software, with spectral matching against the mzCloud MS/MS library based on accurate mass and fragmentation patterns (Christina *et al*., 2021).

Statistical analysis

All the statistical analyses were performed using the SPSS version 24.0. The experimental data were analyzed using analysis of variance (ANOVA) for each incubation period, followed by Tukey's HSD test, with a significance level of 0.05.

RESULTS AND DISCUSSION

Bacterial growth

The growth performance of *L. paracasei* D4 was evaluated in three tofu whey-based formulations (A, B, and C) with varying concentrations of carbon and nitrogen sources, while MRS medium served as the control medium (medium D). Bacterial density of *L. paracasei* D4 (log CFU/mL) was evaluated in all media during five days of incubation at 37 °C, as illustrated in Figure 1. On day 0 (immediately after inoculation), medium B showed the highest viable count of *L. paracasei* D4 (6.65±0.00 log CFU/mL), followed by medium C (6.50±0.00 log CFU/mL), while media A and MRS (medium D) recorded the lowest and statistically similar counts (5.65±0.00 log CFU/mL each). After 24 hours of fermentation, media A and C showed relatively similar and higher viable counts of *L. paracasei* D4 (medium A 8.45±0.03 log CFU/mL and medium C 8.6±0.11 log CFU/mL), while media B and MRS (medium D) were slightly lower and comparable to each other (medium B 7.9±0.09 log CFU/mL and medium D 8.05±0.05 log CFU/mL). By day 3, clear differences between the media became evident, with *L. paracasei* D4 on medium C showing the highest viability (11.85±0.07 log CFU/mL), followed by medium B (10.15±0.12 log CFU/mL), whereas medium A and MRS (medium D) exhibited lower and statistically similar counts medium A (8.65±0.08 log CFU/mL), medium D (8.6±0.13 log CFU/mL). By day 5, the highest viable count was observed in medium C (12.75±0.16 log CFU/mL), while media A, B, and MRS (medium D) reached comparable levels on medium A 11.7±0.04 log CFU/mL, medium B 11.75±0.07 log CFU/mL, medium D 11.65±0.05 log CFU/mL) with no significant differences among them. Overall, the results indicated a growth trend of C>A≈B≈D (MRS). By the end of incubation, the viable count of *L. paracasei* D4 in medium C was approximately 0.85–0.9 log CFU/mL higher than that in the other media.

LAB grew more effectively in tofu whey media supplemented with molasses and skim milk (12.34±0.87 log CFU/mL) than in conventional MRS medium (Maulidiyah *et al*., 2024). Comparable high viable microbial densities have also been reported in fermented systems, with a total plate count (TPC) of

1.7×10¹² CFU/mL reported in naturally fermented tea beverage prepared from banana (*Musa paradisiaca*) and Fuji apple (Lewerissa *et al.,* 2025). Similarly, Fei *et al*. (2022) reported that the tofu-whey-isolated *Lactobacillus amylolyticus* L6 showed strong adaptability to a soymilk-based environment and reached a viable count of approximately 10¹² CFU/mL. In addition, Aydin *et al.* (2026) reported a LAB count of 1.2×10¹² CFU/mL in PFJM, a naturally fermented preparation of meadow grass supplemented with molasses, and noted that molasses supplementation significantly enhanced LAB counts. Papizadeh *et al*. (2020) also reported that *L. paracasei* RPR42 showed its optimal growth when cultured in a medium supplemented with 22.5% sugarcane molasses. LAB can utilize sugars through fermentation, degrade proteins into amino acids, and generate or transform vitamins and other growth-related components (Bintsis, 2018). The synthesis of amino acids can provide advantageous effects to the viability of LAB. Different carbon and nitrogen sources can strongly influence bacterial biomass production. In line with this, a tofu whey-based medium (94.5% tofu whey, 3% molasses, and 2.5% skim milk) developed by Maulidiyah *et al*. (2024) demonstrated higher bacterial growth (12.34±0.87 log CFU/mL) than standard MRS medium (11.48±0.30 log CFU/mL).

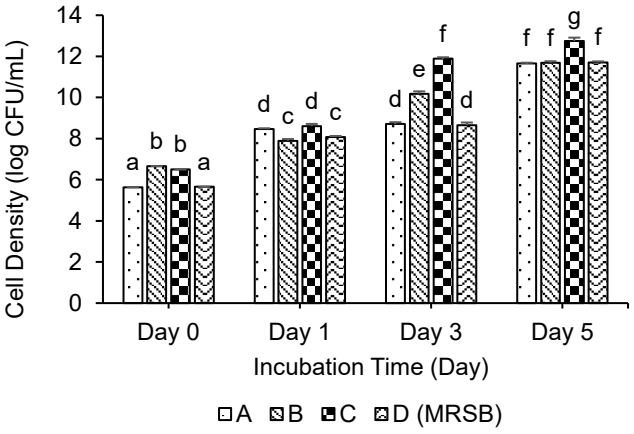


Figure 1. Bacterial growth of *L. paracasei* D4 in all tested media. Different superscript letters indicate significant differences (p<0.05) among treatments at each sampling point, as analyzed by one-way ANOVA and Tukey's post hoc test. Values are presented as mean ± SD

In this study, tofu whey was used as a low-cost and halal alternative to replace the protein and nitrogen-based components commonly found in conventional MRS broth, such as peptone, beef extract, and yeast extract. To evaluate whether tofu whey-based media can provide comparable nutritional support, the proximate composition of the three tofu whey-based media (Media A, B, and C) was analyzed and compared at day 0 and day 5 of fermentation. Tofu whey supplemented with molasses and skim milk can effectively substitute the nutrient sources provided by the standard MRS medium. Its natural composition offers substantial amounts of carbon and nitrogen, which support the metabolic needs of LAB (Maulidiyah *et al*., 2024). Skim milk contains several beneficial components, including lactose as a carbon source, along with peptides, amino acids, vitamins, and essential minerals. Molasses is a good source of carbon to LAB, and it also has other nutrients, such as amino acids and important minerals like manganese and magnesium, which are similar to the mineral profile of MRS (De Souza *et al*., 2015). The properties and composition of the culture medium, as well as the total quality of the culture medium, have a potent effect on LAB growth and metabolic activity.

pH reduction

The alterations of medium pH throughout the 5-day fermentation of *L. paracasei* D4 in tofu whey-based media (Media A, B, and C) and in MRS medium are shown in Figure 2. Initial pH values did not vary greatly among all media at day 0, with average values ranging from 5.34±0.00 to 5.95±0.00. As fermentation progressed, a significant decrease in pH (p<0.05) was observed in all media. On day 1, pH values declined markedly across all media, followed by a further reduction on days 3 and 5. By day 5, the lowest pH values were recorded in medium C (2.84±0.04).

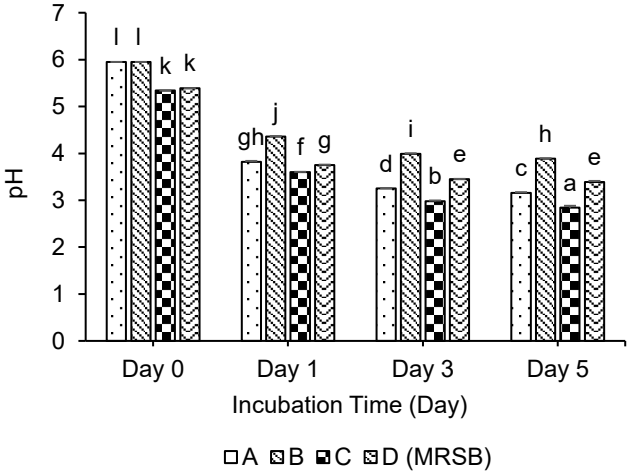


Figure 2. Changes in pH of *L. paracasei* D4 during fermentation across four media (A, B, C, and MRSB) for five days. Different superscript letters indicate significant differences (p<0.05) among treatments at each sampling point, as analyzed by one-way ANOVA and Tukey's post hoc test

As fermentation progresses, carbohydrates in the medium are utilized by *L. paracasei* and converted into organic acids, mainly lactic acid, leading to a decrease in pH (Hashemi *et al*., 2023). Tofu whey-based media supplemented with molasses and skim milk have varying compositions that influence the rate of acidification and overall fermentation activity. Among all tested media, medium C showed the fastest and greatest drop in pH, reaching the lowest final pH of 2.84 by day 5, which was even lower than that recorded in MRS broth. This is likely because medium C contained the highest amount of molasses (7.5%), which provided more fermentable sugars, allowing the bacteria to produce more organic acids during fermentation. Although tofu whey-based media may behave differently from MRS broth in terms of acidity, the data clearly show that the higher sugar content in medium C led to more active fermentation and a faster drop in pH, rather than a slower one. Tofu whey has a different nutritional composition, which can influence the efficiency of sugar utilization, microbial metabolic activity, and the extent of acidification. Moreover, maintaining a lower pH is crucial for preserving the antibacterial activity of *L. paracasei,* thereby enhancing its probiotic efficacy and product stability (Rocchetti *et al*., 2021). It is known that an optimal acidic environment increases the antimicrobial activity of the lactic acid, suppressing growth of unwanted microorganisms and promoting a favorable environment for probiotic organisms (Tachedjian *et al*., 2017).

Proximate composition changes

The proximate composition of tofu whey-based media (Media A, B, and C) was measured on day 0 and day 5 of fermentation, as shown in Table 1. Protein content changed only slightly during fermentation, with a slight increase observed in Media C on day 5 of fermentation (from 0.22 to 0.37%), while Media A and B remained almost the same. The slight increase in protein content observed in medium C may be attributed to the accumulation of bacterial biomass during fermentation, as proximate analysis of the fermented medium also captures proteins derived from microbial cells. Fat content was low in all media on day 0 and on day 5 of fermentation and changed only slightly during fermentation with values ranging from 0.01 to 0.07%. Water content was high in all media and increased slightly on day 5 of fermentation. Ash content showed minimal variation between day 0 and day 5 of fermentation, indicating stable mineral levels. However, carbohydrate content decreased during fermentation from 0.96-0.99% at day 0 to 0.52–0.69% at day 5, showing a reduction of 0.28–0.44%, with the highest decrease observed in medium C. As medium C contained the highest molasses concentration (7.5%) among the three alternative media, resulting in the highest initial carbohydrate content (0.90%) at day 0. This higher carbohydrate availability likely supported greater LAB growth, as sugars are the primary energy source for LAB metabolism. The greatest carbohydrate reduction in medium C (0.90% to 0.52%) between day 0 and day 5 further confirms more active fermentation compared to media A and B.

Reduction in carbohydrate level during fermentation was because of direct utilization by the microbes. LAB, especially *L. paracasei*, break down simple sugars into lactic acid and other metabolic by-products causing a large loss of carbohydrates (Cui *et al*., 2021). Changes in protein content during fermentation are because of microbial activity, as LAB break down proteins into smaller components such as peptides and amino acids, which are then used to support their growth and metabolism. Although LAB are generally known to utilize carbohydrates and proteins, several studies have shown that certain strains of LAB are also able to metabolize lipids, either as an energy source or by converting them into bioactive compound (Wang *et al*., 2022). As moisture plays a key role in fermentation rate and product stability, maintaining appropriate levels is important to optimize fermentation and ensure consistent product quality (Sharma *et al*., 2020).

Antibacterial activity

Antibacterial activity was assessed using the cell-free supernatant of *L. paracasei* D4 against four bacteria, including *S. aureus* ATCC 29213*, B. cereus* ATCC 11778*, S.* Typhimurium TRD1, and *E. coli* 25922. The inhibitory effects of both non-neutralized and neutralized CFS samples across these strains are illustrated in Figure 3a–3d. Across all tested bacteria, antibacterial activity increased with fermentation time, with the highest inhibition observed on day 5. The tofu whey-based medium (medium C) containing 90% tofu whey, 7.5% molasses, and 2.5% skim milk consistently showed the strongest inhibition compared to the other formulations and the MRS control. Media A and B showed lower inhibition, especially at the early stage of fermentation, while the MRS medium showed moderate activity that increased over time.

Table 1. Proximate composition of tofu-based media (Media A, B, and C) at day 0 and day 5

Parameters (%)	Day 0			Day 5		
	Medium A	Medium B	Medium C	Medium A	Medium B	Medium C
Protein	0.12	0.28	0.22	0.13	0.26	0.37
Fat	0.07	0.02	0.01	0.04	0.02	0.06
Water	98.45	98.26	98.42	98.77	98.61	98.62
Ash	0.37	0.47	0.45	0.38	0.42	0.43
Carbohydrate	0.99	0.97	0.96	0.68	0.69	0.52

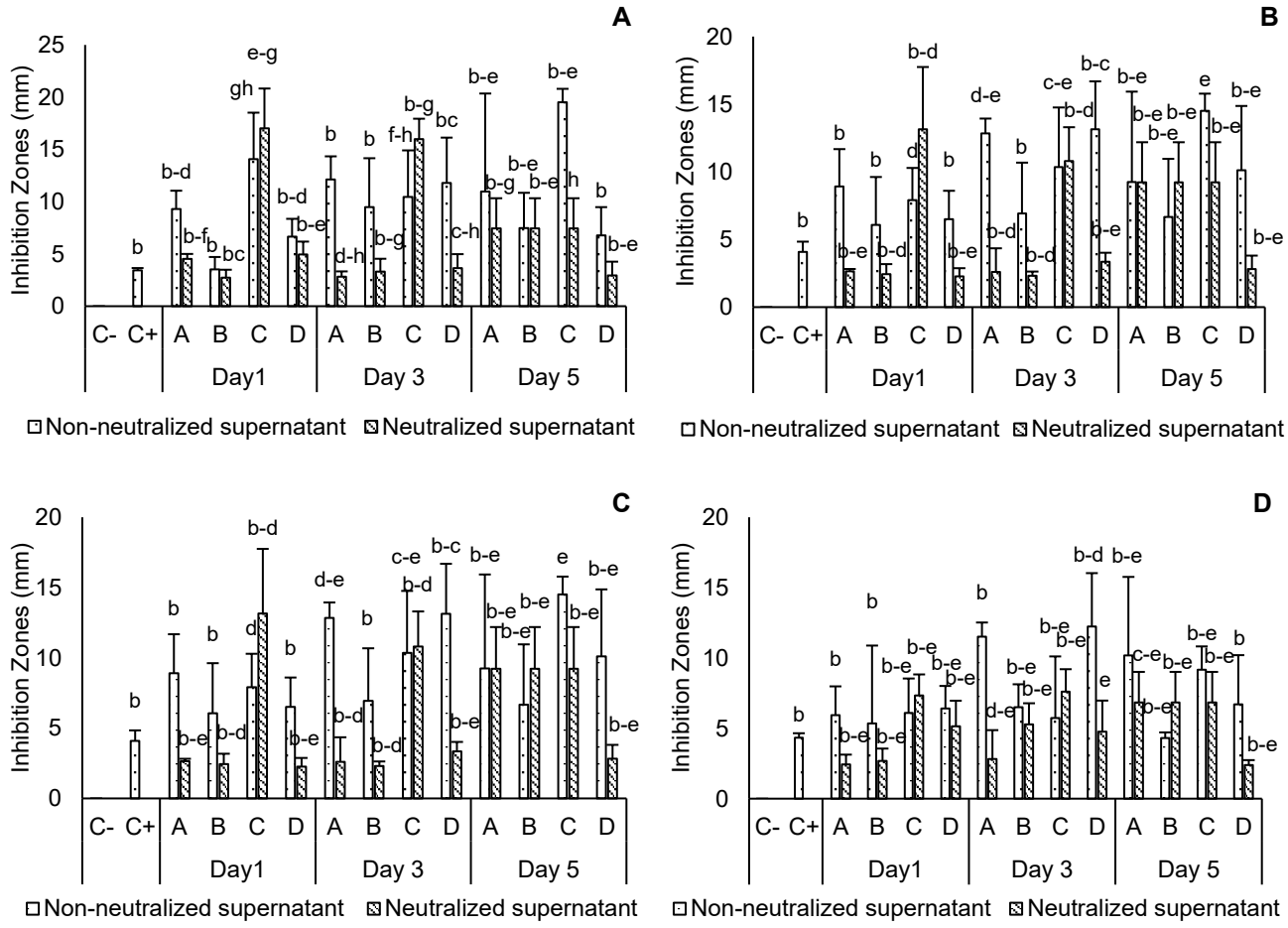


Figure 3. Antibacterial activity of *L. paracasei* D4 cell-free supernatants produced in tofu whey-based media and MRS broth against *S. aureus ATCC 29213* (a), *B. cereus* ATCC 11778 (b), *E. coli* ATCC 25922 (c), and *S.* Typhimurium TRD1 (d). Antibacterial activity was assessed using non-neutralized and pH-neutralized (pH 6.0-6.5) supernatants collected on day 1, 3, and 5 of fermentation. C- and C+ represent the negative (saline water) and positive controls (chloramphenicol), respectively**.** Values are presented as mean ± SD, with different letters indicating significantly differences (p<0.05)

In all treatments, non-neutralized cell-free supernatants showed stronger inhibition than neutralized samples, indicating that organic acids play a major role in the antimicrobial effect. However, inhibition was still observed after pH neutralization, particularly in later stages of fermentation. This suggests that the antibacterial activity is not only due to organic acids, but also involves other compounds such as bacteriocin-like inhibitory substances (BLIS). Overall, these results show that the tofu whey-based medium supports the production of both acid-dependent and pH-independent antimicrobial compounds by *L. paracasei* D4, contributing to its activity against both Gram-positive and Gram-negative bacteria.

Lactic acid is predominantly produced by *L. paracasei* as the main organic acid during fermentation. The accumulation of this acid lowers the pH of the medium, creating an acidic environment that can inhibit the growth of various pathogenic microorganisms (Chang *et al*., 2021). Previous studies have also reported that the growth of *L.*

paracasei in different culture media is closely associated with lactic acid production and antibacterial activity (Beret *et al*., 2021). Besides organic acids, *L. paracasei* has also been reported to produce bacteriocins, which are pH-independent antimicrobial compounds capable of inhibiting the growth of pathogenic bacteria and contributing to biopreservation (Arena *et al*., 2016). The antimicrobial potential of *L. paracasei* may be further enhanced when grown in alternative media derived from agro-industrial by-products, such as tofu whey, which support bacterial growth and metabolic activity, and may be associated with the presence of antimicrobial-related metabolites (Mutmainna *et al*., 2021).

Previous studies have shown that non-neutralized CFS generally exhibit stronger antimicrobial activity due to the combined effects of organic acids and low pH conditions (Arrioja-Bretón *et al*., 2020). In contrast, neutralization reduces antibacterial activity by diminishing the effect of organic acids, although some residual activity may remain due to bacteriocin production (Hussain *et al*., 2021). *L. paracasei* D4 shows strong inhibitory activity against *B. cereus* and *S. aureus*, suggesting that its antimicrobial activity is not only due to organic acids but may also involve other metabolites, such as bacteriocins. Environmental factors such as fermentation time and temperature can influence organic acid production and microbial metabolism, which may affect the antimicrobial activity of *L. paracasei* (Arena *et al*., 2016). Previous studies have shown that tofu whey-based media can effectively support the growth of *L. paracasei* D4 and may even exhibit antibacterial activity comparable to, or higher than, that of conventional media (Maulidiyah *et al*., 2024). The use of *L. paracasei* D4 in alternative fermented substrates provides both probiotic benefits and natural antimicrobial effects, thereby enhancing food safety.

Identity of bacteriocin-related metabolites using LC-HRMS

LC-HRMS analysis of the fermentation extracts identified a total of 209 metabolites across the different medium, including organic acids and peptide-related compounds. Among these, 159 compounds were classified as organic acids, while 50 compounds were peptide-related molecules, including various cyclic dipeptides. Among the detected compounds, nine compounds were identified as bacteriocin-like or antibacterial-related substances that are showed in Table 2. Most of the detected metabolites were cyclic peptides and diketopiperazine derivatives, which are widely reported antimicrobial compounds produced by LAB during fermentation. Indole-3-lactic acid was detected in all alternative media formulations, indicating that its production is a stable metabolic feature of *L. paracasei* D4 and is not strongly influenced by media composition. In contrast, other compounds, including Diprotin A and cyclo (D-Pro-D-Leu), were detected only in specific formulations, suggesting that peptide production may vary depending on the nutrient composition of the medium.

The identification of antimicrobial-related metabolites in this study is consistent with previous reports that LAB can generate bioactive compounds with potential bactericidal effects. Several cyclic dipeptides, including cyclo(Ile-Ser), cyclo(L-Ala-L-Tyr), and cyclo(Pro-Val-Val), were identified across different media, with medium C showing a greater diversity of antimicrobial-related peptides. These compounds have been reported to contribute to antibacterial activity by interfering with bacterial cell membranes and metabolic functions. LC-HRMS analysis also identified other antimicrobial-related metabolites, including Indole-3-lactic acid, Diprotin A, and trans-3-Indoleacrylic acid, indicating that *L. paracasei* D4 can produce a diverse range of bioactive compounds with potential antimicrobial properties. The presence of these metabolites supports the observed antibacterial activity of the cell-free supernatants and suggests that tofu whey-based media, particularly medium C, can promote the production of antimicrobial compounds by *L. paracasei* D4. Cyclo(Pro-Val-Val) is a cyclic dipeptide that has been associated with antibacterial activity by interfering with the growth of competing bacteria, thereby contributing to the antimicrobial potential of microbial fermentation products (Brudzynski, 2021). Indole-3-lactic acid, a tryptophan-derived metabolite well documented in *L. paracasei* (Si *et al*., 2023), was detected in media A, B, and C, further supporting its production by *L. paracasei* D4 during fermentation. Other compounds were also identified with varying retention time and m/z values, including Diprotin A ($C_{17}H_{31}N_3O_4$) and cyclo(Ile-Ser) ($C_9H_{16}N_2O_3$), indicating structural heterogeneity that can affect their antimicrobial functions in the case of certain LAB strains (Liu *et al*., 2022). Such data can be used to estimate the potential of the LAB strains to produce bacteriocins more intensely (Sharma *et al*., 2020).

Table 2. Antimicrobial and bacteriocin-related peptide metabolites identified by liquid chromatography-high resolution mass spectrometry (LC-HRMS) analysis

No.	Compound Name	Molecular Formula	m/z	RT (min)	Media	References
1	Indole-3-lactic acid	$C_{11}H_{11}NO_3$	206.08	8.97	A, B, C	(Yao *et al*., 2024)
2	Diprotin A	$C_{17}H_{31}N_3O_4$	342.24	8.80	B, D	(Ben Lagha *et al*., 2017)
3	Trans-3-indoleacrylic acid	$C_{11}H_9NO_2$	188.07	2.32	A, B, C, D	(Fabricio *et al*., 2025)
4	Cyclo(Ile-Ser)	$C_9H_{16}N_2O_3$	201.12	0.95	A, B, C	(Shin *et al*., 2023a)
5	Cyclo(D-Pro-0D-Leu)	$C_{11}H_{18}N_2O_2$	211.14	8.22	B, D	(Ben Lagha *et al*., 2017)
6	Cyclo(L-Ala-L-Tyr)	$C_{12}H_{14}N_2O_3$	235.11	0.67	B	(Shin *et al*., 2023b)
7	Cycloserine diketopiperazine	$C_6H_{12}N_4O_4$	103.05	13.00	A, B, C	(Kang *et al*., 2015)
8	1,8-diazacyclotetradecane-2,7-dione	$C_{12}H_{22}N_2O_2$	227.17	8.43	A	(Câmara *et al*., 2020)
9	Cyclo(Pro-Val-Val)	$C_{15}H_{25}N_3O_3$	296.20	2.90	C	(Brudzynski, 2021)

Overall, LC-HRMS analysis indicated the presence of antimicrobial-related metabolites that may support the observed inhibition, and medium C showing a higher diversity of peptide-based compounds consistent with stronger inhibitory activity. These findings suggest that both organic acids and peptide-derived compounds play a role in the observed inhibition. However, this study has several limitations. The compounds were identified based on database matching without further structural confirmation, and their individual contributions were not quantified. In addition, the study was limited to *in vitro* conditions. Further work is required to better understand the specific roles and practical potential of these compounds.

CONCLUSION

This study shows that tofu whey can be used as an effective and low-cost alternative medium for the growth of *L. paracasei* D4 and its antibacterial activity. Among the tested formulations, medium C: tofu whey (90%), molasses (7.5%), skim milk (2.5%), consistently gave the best results in terms of bacterial growth, acid production, and inhibition against pathogenic bacteria. The results indicate that the observed antimicrobial effect was mainly associated with organic acid production, although peptide-based compounds may also play a role. Overall, tofu whey with composition of tofu whey (90%), molasses (7.5%), skim milk (2.5%) shows strong potential for sustainable probiotic cultivation and natural antimicrobial production. Furthermore, the stability of tofu whey-based media during storage was not investigated in this study and should be addressed in future research to better evaluate its practical feasibility for industrial application.

ACKNOWLEDGMENTS

We would like to sincerely thank the Kemitraan Negara Berkembang (KNB) Scholarship, supported by the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, for providing financial support during this study. We also appreciate the Faculty of Mathematics and Natural Sciences, Universitas Brawijaya, for the Internal Research Grant (No. 02115.20/UN10.F0901/B/PG/2025).

REFERENCES

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., & Kuipers, O. P. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Applied Microbiology and Biotechnology*, *100*(7), 2939–2951. https://doi.org/10.1007/s00253-016-7343-9

Arena, M. P., Silvain, A., Normanno, G., Grieco, F., Drider, D., Spano, G., & Fiocco, D. (2016). Use of *Lactobacillus plantarum* strains as a bio-control strategy against food-borne pathogenic microorganisms. *Frontiers in Microbiology*, *7*, 464. https://doi.org/10.3389/fmicb.2016.00464

Arrioja-Bretón, D., Mani-López, E., Palou, E., & López-Malo, A. (2020). Antimicrobial activity and storage stability of cell-free supernatants from lactic acid bacteria and their applications with fresh beef. *Food Control*, *115*, 107286. https://doi.org/10.1016/j.foodcont.2020.107286

AOAC International. (2019). *Official Methods of Analysis of AOAC International* (21st ed.). AOAC International.

Aydin, S. S., Avci, M., Kirar, N., Oruç, A., Savrunlu, M., & Daş, A. (2026). Investigation of the effects of using natural fermented lactic acid bacteria as probiotics on fattening performance, blood parameters, and intestinal microflora in broiler chickens under heat stress. *Veterinary Sciences, 13*(5), 488. https://doi.org/10.3390/vetsci13050488

Ben Lagha, A., Haas, B., Gottschalk, M., & Grenier, D. (2017). Antimicrobial potential of bacteriocins in poultry and swine production. *Veterinary Research*, *48*(1), 22. https://doi.org/10.1186/s13567-017-0425-6

Beret, M. V., Peralta, G. H., Vera-Candioti, L., Wolf, I. V., Sánchez, R., Hynes, E. R., & Bergamini, C. V. (2021). Culture media based on effluent derived from soy protein concentrate production for *Lacticaseibacillus paracasei* 90 biomass production: Statistical optimisation, mineral characterization, and metabolic activities. *Antonie van Leeuwenhoek*, *114*(12), 2047–2063. https://doi.org/10.1007/s10482-021-01660-1

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiology*, *4*(4), 665–684. https://doi.org/10.3934/microbiol.2018.4.665

Brudzynski, K. (2021). Honey as an ecological reservoir of antibacterial compounds produced by antagonistic microbial interactions in plant nectars, honey and honey bee. *Antibiotics*, *10*(5), 551. https://doi.org/10.3390/antibiotics10050551

Câmara, G. A., Nishiyama-Jr, M. Y., Kitano, E. S., Oliveira, U. C., da Silva, P. I. J., Junqueira-de-Azevedo, I. L., & Tashima, A. K. (2020). A multiomics approach unravels new toxins with possible *in silico* antimicrobial, antiviral, and antitumoral activities in the venom of *Acanthoscurria rondoniae*. *Frontiers in Pharmacology, 11,* 1075. https://doi.org/10.3389/fphar.2020.01075

Chang, H. M., Foo, H. L., Loh, T. C., Lim, E. T. C., & Abdul Mutalib, N. E. (2021). Comparative studies of inhibitory and antioxidant activities, and organic acids compositions of postbiotics produced by probiotic *Lactiplantibacillus plantarum* strains isolated from Malaysian foods. *Frontiers in Veterinary Science, 7,* 602280. https://doi.org/10.3389/fvets.2020.602280

Christina, Y. I., Nafisah, W., Fitri Atho'illah, M., Rifa'i, M., Widodo, N., & Djati, M. S. (2021). Anti-breast cancer potential activity of *Phaleria macrocarpa* (Scheff.) Boerl. leaf extract through *in silico* studies. *Journal of Pharmacy & Pharmacognosy Research, 9*(6), 824–845. https://doi.org/10.56499/jppres21.1092_9.6.824

Cui, Y., Wang, M., Zheng, Y., Miao, K., & Qu, X. (2021). The carbohydrate metabolism of *Lactiplantibacillus plantarum*. *International Journal of Molecular Sciences, 22*(24), 13452. https://doi.org/10.3390/ijms222413452

De Souza, R. B., De Menezes, J. A. S., De Souza, R. D. F. R., Dutra, E. D., & De Morais Jr, M. A. (2015). Mineral composition of the sugarcane juice and its influence on the ethanol fermentation. *Applied Biochemistry and Biotechnology, 175*(1), 209–222. https://doi.org/10.1007/s12010-014-1258-7

Dos Santos, F. P. dos, Magalhães, D., Nascimento, J. dos S., & Ramos, G. L. P. A. (2021). Use of products of vegetable origin and waste from hortofruticulture for alternative culture media. *Food Science and Technology International, 28*(7), 545–555. https://doi.org/10.1590/fst.00621

Fabricio, M. F., Schmidt, L., Rother, P. D. H., Augusti, P. R., Candido, F., Wagner, R., Anzanello, M. J., Rodrigues, E., & Ayub, M. A. Z. (2025). Targeted metabolomics of phenolic and volatile compounds during the fermentation of a potential probiotic tofu whey beverage. *Food Chemistry, 478,* 143689. https://doi.org/https://doi.org/10.1016/j.foodchem.2025.143689

Fei, Y., Huang, L., Wang, H., Liang, J., Liu, G., & Bai, W. (2022). Adaptive mechanism of *Lactobacillus amylolyticus* L6 in soymilk environment based on metabolism of nutrients and related gene-expression profiles. *Food Science & Nutrition, 10*(5), 1548–1563. https://doi.org/10.1002/fsn3.2779

Ginting, E., Elisabeth, D. A. A., Khaitenmidah, A., Rinaldi, J., Ambarsari, I., & Antarlina, S. S. (2024). The nutritional and economic potential of tofu dreg (okara) and its utilization for high protein food products in Indonesia. *Journal of Agriculture and Food Research, 16,* 101147. https://doi.org/10.1016/j.jafr.2024.101147

Giulianetti de Almeida, M. P., Mockaitis, G., & Weissbrodt, D. G. (2023). Got whey? Sustainability endpoints for the dairy industry through resource biorecovery. *Fermentation*, *9*(10), 897. https://doi.org/10.3390/fermentation9100897

Hadidi, M., Aghababaei, F., González-Serrano, D. J., Goksen, G., Trif, M., McClements, D. J., & Moreno, A. (2024). Plant-based proteins from agro-industrial waste and by-products: Towards a more circular economy. *International Journal of Biological Macromolecules*, *258,* 129576. https://doi.org/10.1016/j.ijbiomac.2024.129576

Hashemi, S. M. B., Roohi, R., Akbari, M., Di Natale, A., & Conte, F. (2023). Inactivation of

foodborne pathogens by *Lactiplantibacillus* strains during meat fermentation: Kinetics and mathematical modelling. *Foods*, *12*(17), 3150. https://doi.org/10.3390/foods12173150

Hussain, N., Tariq, M., Saris, P. E. J., & Zaidi, A. (2021). Evaluation of the probiotic and postbiotic potential of lactic acid bacteria from artisanal dairy products against pathogens. *The Journal of Infection in Developing Countries,* *15*(01), 102–112. https://doi.org/10.3855/jidc.13404

ISO. (2013). *Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 1: Colony count at 30°C by the pour plate technique* (ISO Standard No. 4833-1:2013). International Organization for Standardization. https://www.iso.org/standard/53728.html

Kang, H. K., Seo, C. H., & Park, Y. (2015). Marine peptides and their anti-infective activities. *Marine Drugs*, *13*(1), 618–654. https://doi.org/10.3390/md13010618

Kilama, J., Yakir, Y., Shaani, Y., Adin, G., Kaadan, S., Wagali, P., Sabastian, C., Ngomuo, G., & Mabjeesh, S. J. (2023). Chemical composition, *in vitro* digestibility, and storability of selected agro-industrial by-products: Alternative ruminant feed ingredients in Israel. *Heliyon, 9*(3), e14581. https://doi.org/10.1016/j.heliyon.2023.e14581

Kolesovs, S., & Semjonovs, P. (2020). Production of bacterial cellulose from whey—Current state and prospects. *Applied Microbiology and Biotechnology*, *104,* 7723–7730. https://doi.org/10.1007/s00253-020-10769-x

Liu, A., Yan, X., Shang, H., Ji, C., Zhang, S., Liang, H., Chen, Y., & Lin, X. (2022). Screening of *Lactiplantibacillus plantarum* with high stress tolerance and high esterase activity and their effect on promoting protein metabolism and flavor formation in Suanzhayu, a Chinese fermented fish. *Foods*, *11*(13), 1932. https://doi.org/10.3390/foods11131932

Lewerissa, K. B., Rahardjo, M., Budi, R. N., Jovanka, R., & Carouline, B. (2025**).** Quality of tea fermented drink from natural fermentation of banana (*Musa paradisiaca*) and Fuji apple (*Malus domestica*). *Journal of Food, Culinary, and Nutrition, 1*(2)*,* 67–77. https://doi.org/10.24167/jfcn.v1i2.12524

Maselli, L., & Hekmat, S. (2016). Microbial vitality of probiotic milks supplemented with cereal or pseudocereal grain flours. *Journal of Food Research*, *5*(5), 41–49. https://doi.org/10.5539/jfr.v5n5p41

Maulidiyah, H., Mustafa, I., Jatmiko, Y. D., & Ibrahim, S. A. (2024). Tofu whey-based media for probiotic *Lactiplantibacillus plantarum* D4 as a halal starter culture. *Indonesian Journal of Halal Research*, *6*(1), 26–35. https://doi.org/10.15575/ijhar.v6i1.30588

Miranda, M. H., & Nader-Macías, M. E. F. (2023). Low-cost culture media designed for biomass production of beneficial lactic acid bacteria for their inclusion in a formula to treat bovine reproductive infections. *FEMS Microbiology Letters*, *370,* 1–6. https://doi.org/10.1093/femsle/fnad033

Mutmainna, A., Arief, I. I., & Budiman, C. (2021). The growth and production of antimicrobial compounds from *Lactobacillus plantarum* IIA-1A5 on cheese whey medium. *Journal of the Indonesian Tropical Animal Agriculture*, *46*(2), 173–184. https://doi.org/10.14710/jitaa.46.2.173-184

Papizadeh, M., Rohani, M., Nahrevanian, H., Hosseini, S. N., Shojaosadati, S. A., & Pourshafie, M. R. (2020). Using various approaches of design of experiments for high cell density production of the functionally probiotic *Lactobacillus plantarum* strain RPR42 in a cane molasses-based medium. *Current Microbiology, 77,* 1756–1766. https://doi.org/10.1007/s00284-020-01990-1

Rocchetti, M. T., Russo, P., Capozzi, V., Drider, D., Spano, G., & Fiocco, D. (2021). Bioprospecting antimicrobials from *Lactiplantibacillus plantarum*: Key factors underlying its probiotic action. *International Journal of Molecular Sciences*, *22*(21), 12076. https://doi.org/10.3390/ijms222112076

Sharma, R., Garg, P., Kumar, P., Bhatia, S. K., & Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation*, *6*(4), 106. https://doi.org/10.3390/fermentation6040106

Shin, M. K., Park, H.-R., Hwang, I.-W., Bu, K.-B., Jang, B.-Y., Lee, S.-H., Oh, J. W., Yoo, J. S., & Sung, J.-S. (2023). In silico-based design of a hybrid peptide with antimicrobial activity against multidrug-resistant *Pseudomonas aeruginosa* using a spider toxin peptide. *Toxins*, *15*(12), 668. https://doi.org/10.3390/toxins15120668

Si, Q., Wang, Z., Liu, W., Liu, M., Ge, G., Jia, Y., & Du, S. (2023). Influence of cellulase or *Lactiplantibacillus plantarum* on the ensiling performance and bacterial community in mixed silage of alfalfa and *Leymus chinensis*.

Microorganisms, *11*(2), 426. https://doi.org/10.3390/microorganisms11020426

Syahniar, R., Wahid, M., & Yasmon, A. (2026). The role of *Lacticaseibacillus paracasei* as a probiotic in health. *Journal of Pure and Applied Microbiology*, *20*(1), 150. https://doi.org/10.22207/JPAM.20.1.15

Tachedjian, G., Aldunate, M., Bradshaw, C. S., & Cone, R. A. (2017). The role of lactic acid production by probiotic *Lactobacillus* species in vaginal health. *Research in Microbiology*, *168*(9), 782–792. https://doi.org/10.1016/j.resmic.2017.04.001

Tindjau, R., Chua, J.-Y., & Liu, S.-Q. (2023a). Growth and metabolic behaviour of probiotic *Bifidobacteria longum* subsp. *longum* in minimally supplemented soy (tofu) whey. *Future Foods*, *8,* 100262. https://doi.org/10.1016/j.fufo.2023.100262

Tindjau, R., Chua, J.-Y., & Liu, S.-Q. (2023b). Growth, substrate, and metabolite changes of probiotic *Bifidobacterium animalis* subsp. *lactis* in soy (tofu) whey. *Fermentation, 9*(12), 1014. https://doi.org/10.3390/fermentation9121014

Valle Vargas, M. F., Villamil Diaz, L. M., Ruiz Pardo, R. Y., & Quintanilla Carvajal, M. X. (2024). Design of an agro-industrial by-products-based media for the production of probiotic bacteria for fish nutrition. *Scientific Reports*, *14*(1), 17955. https://doi.org/10.1038/s41598-024-68783-z

Wang, D., Cheng, F., Wang, Y., Han, J., Gao, F., Tian, J., Zhang, K., & Jin, Y. (2022). The changes occurring in proteins during processing and storage of fermented meat products and their regulation by lactic acid bacteria. *Foods*, *11*(16), 2427. https://doi.org/10.3390/foods11162427

Wang, S.-K., Wang, X., Miao, J., & Tian, Y.-T. (2018). Tofu whey wastewater is a promising basal medium for microalgae culture. *Bioresource Technology*, *253,* 79–84. https://doi.org/10.1016/j.biortech.2018.01.011

Wang, W., & Wang, H. (2014). The effect of lactic acid bacteria in food and feed and their impact on food safety. *International Journal of Food Engineering*, *10*(2), 203–210. https://doi.org/10.1515/ijfe-2014-0010

Yang, H., Hao, L., Jin, Y., Huang, J., Zhou, R., & Wu, C. (2024). Functional roles and engineering strategies to improve the industrial functionalities of lactic acid bacteria during food fermentation. *Biotechnology Advances*, *74,* 108397. https://doi.org/10.1016/j.biotechadv.2024.108397

Yao, M., Cao, J., Zhang, L., Wang, K., Lin, H., Qin, L., Zhang, Q., Qu, C., Miao, J., & Xue, C. (2024). Indole-3-Lactic Acid derived from *Lacticaseibacillus paracasei* inhibits *Helicobacter pylori* infection via destruction of bacteria cells, protection of gastric mucosa epithelial cells, and alleviation of inflammation. *Journal of Agricultural and Food Chemistry, 72*(28), 15725–15739. https://doi.org/10.1021/acs.jafc.4c02868

Zhang, S., Wang, J., & Jiang, H. (2020). Microbial production of value-added bioproducts and enzymes from molasses, a by-product of sugar industry. *Food Chemistry, 346,* 128860. https://doi.org/10.1016/j.foodchem.2020.128860