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CHARACTERIZATION AND PROBIOTIC POTENTIAL OF LACTIC ACID BACTERIA FROM WEST SUMATRA DADIH (TRADITIONAL FERMENTED BUFFALO MILK) AS FUNCTIONAL FOOD STARTER CANDIDATES

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ABSTRACT

Dadih is a spontaneously fermented buffalo milk from West Sumatra, made in bamboo tubes capped with banana leaves and driven by indigenous lactic acid bacteria (LAB) with probiotic potential. This study aimed to characterize dadih from three production centers, isolate LAB, test probiotic properties, and select the best isolate as a functional food starter candidate. Total LAB was enumerated by the pour plate method, pH and titratable acidity were measured, and six isolates were tested for tolerance to pH 2.0 and 0.3% bile salts, antagonism against Escherichia coli and Salmonella sp., and auto-aggregation. Data were analyzed by ANOVA and Duncan's test at 5%. Total LAB ranged from 8.82 to 9.21 log CFU/mL with pH 3.94–4.31. All isolates were Gram-positive and catalase-negative. Isolate I-3 performed best, with 91.3% survival at pH 2.0, 93.4% in bile salts, inhibition zones of 13.1 mm (E. coli) and 12.0 mm (Salmonella), and 88.5% auto-aggregation. Isolate I-3 is recommended as a dadih starter candidate pending molecular identification and safety assessment.

Keywords: dadih; lactic acid bacteria; probiotic; functional food; West Sumatra

1. INTRODUCTION

Traditional fermented foods represent a body of local knowledge that frequently aligns with modern food science findings. In West Sumatra, one notable example is dadih (dadiah), a spontaneously fermented buffalo milk product. Dadih is prepared by pouring fresh buffalo milk into bamboo tubes sealed with banana leaves, which are then left at room temperature for approximately two days (Aritonang et al., 2022). No starter cultures are added; the fermentation process is entirely driven by indigenous lactic acid bacteria (LAB) originating from the milk and the production environment. This simple process yields a firm, yogurt-like product long consumed by the Minangkabau community.

Interest in probiotic-based functional foods continues to grow. A widely accepted definition describes probiotics as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host—a definition reaffirmed in the ISAPP consensus statement (Hill et al., 2014). For a LAB isolate to be considered a viable probiotic candidate, it must at least tolerate gastric acidity and bile salts, inhibit pathogen growth, and possess cell surface properties conducive to colonization, such as auto-aggregation ability.

Several recent studies indicate that dadih is a promising source of LAB. Dadih collected from five dairy farmers in Tanah Datar contained total LAB counts of 7×10^8 to 23×10^8 CFU/mL; the isolates were Gram-positive rod-shaped bacteria resistant to acid and bile salts, exhibited inhibitory activity against pathogenic bacteria, and one selected isolate closely resembled Lactobacillus casei (Aritonang et al., 2022). Isolates from other West Sumatran dadih, identified via 16S rRNA sequencing, were found to be Lactobacillus fermentum with catalase-negative and homofermentative characteristics (Amelia et al., 2020). Dadih's potential has also been evaluated from other perspectives: isolate DK1 from dadih exhibited resistance to pH 2–4, temperatures of 25–45 °C, and 0.3% bile salts,

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while effectively inhibiting Salmonella sp. and Escherichia coli, leading to the testing of its metabolites as a beef biopreservative at 4 °C for nine days (Yuliana et al., 2023). The quality and potential of dadih from specific regions, such as dadiah kapau from Agam Regency, have also been reported as health-promoting probiotic sources (Roza et al., 2022).

Lactic acid bacteria play a crucial role in fermented foods by producing lactic acid and other organic acids that lower pH, as well as antimicrobial compounds such as hydrogen peroxide and bacteriocins that suppress spoilage organisms and pathogens. In the context of functional foods, these characteristics form the foundation for utilizing LAB, both as live probiotics and as natural biopreservatives. Strain selection must not rely solely on isolation origin, but must be grounded in measurable properties, including gastrointestinal survival, pathogen antagonism, and safety.

Buffalo milk, the primary raw material for dadih, represents a unique local resource. Developing products based on fermented buffalo milk aligns with efforts to diversify local animal-derived foods and strengthen regional food identities. Amid rising consumer interest in fermented foods and gut health, dadih offers distinct cultural value that imported products cannot easily replicate; thus, scientific research explaining its LAB characteristics can bolster its market positioning.

Nevertheless, the characteristics of dadih LAB vary significantly across production centers due to spontaneous fermentation influenced by variations in milk, bamboo containers, banana leaves, and individual farmer practices. While this variability means findings from one study may not apply universally, it presents an opportunity to discover region-specific superior strains. Furthermore, comparative studies evaluating multiple production centers using a standardized testing panel (acid resistance, bile salt tolerance, pathogen antagonism, and auto-aggregation) within a single investigation remain limited. Such comprehensive evaluations facilitate objective isolate selection based on composite rankings.

From a preservation perspective, traditional dadih faces notable challenges. Production remains largely small-scale, unstandardized, and characterized by a short shelf life. Identifying superior LAB strains enables the development of controlled starter cultures, allowing dadih to be produced with greater consistency and safety without sacrificing its cultural identity. Traditional practices regarding bamboo, banana leaves, and two-day fermentation can thus be integrated with modern quality control standards.

Recent reviews emphasize that while dadih possesses substantial value, it also faces key operational constraints. Fresh dadih harbors active LAB populations around 10^8 CFU/g with reported antimicrobial, antioxidant, antimutagenic, hypocholesterolemic, and immunomodulatory properties; however, traditional production is hindered by limited buffalo milk supply, lack of standardization due to spontaneous fermentation, safety concerns arising from the absence of thermal treatment, and limited shelf life and consumer acceptability (Herlina & Setiarto, 2024). Other reviews highlight that dadih is typically produced without heat treatment, suggesting that safety and standardization issues should be addressed through pasteurization and incubator development (Arnold et al., 2021). Raw material constraints are equally evident: studies on local Indonesian buffalo biodiversity underscore the current status, challenges, and development opportunities for buffalo populations (Prihandini et al., 2023), which directly govern milk availability for dadih production.

The diversity of dadih microbial communities underlines the necessity of strain selection. Dadih produced via back-slopping or spontaneous fermentation across two West Sumatran regions displayed distinct LAB diversity (Wirawati et al., 2019), and dadih microbiota composition has been profiled using molecular approaches (Venema & Surono, 2019). Beyond microbiota, the nutritional composition of dadih varies by region, as reported for dadih from Lintau (Alzahra et al., 2021), Tanjung Bonai, Tanah Datar (Aritonang et al., 2021), and Kapau, Agam Regency (Putri et al., 2021). These variations demonstrate that each production center possesses unique characteristics worthy of investigation.

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From a functional standpoint, dadih-derived LAB have been linked to diverse benefits. Probiotic isolates from West Sumatran traditional fermented foods have been reported to inhibit E. coli, Staphylococcus aureus, and Salmonella enteritidis (Harnentis et al., 2020), while other isolates synthesize γ-aminobutyric acid (GABA), a key inhibitory neurotransmitter (Harnentis et al., 2019). The antioxidant activity of soluble dadih proteins across several regencies has been documented (Kusumaningtyas & Utami, 2020), and dadih administration in aging rats reduced renal malondialdehyde levels (Harun et al., 2020), while pancreatic histopathology in diabetic mice improved following dadih treatment (Armal & Amien, 2023). Lactobacillus casei subsp. casei R-68 from dadih reduced procarcinogenic enzyme activities in rat feces (Pato et al., 2019). In pediatric studies, supplementation with dadih-derived L. plantarum IS-10506 enhanced humoral immune responses and zinc status (Surono et al., 2014) as well as fecal sIgA levels (Kusumo et al., 2019), while the complete genome of L. plantarum Dad-13 was established to support its probiotic potential (Rahayu et al., 2024).

These scientific advancements have yet to translate into widespread application at the producer level. Spontaneous fermentation carries inherent risks of spoilage and pathogenic contamination if sanitation is not strictly controlled, illustrating that fermented foods possess a dual nature of benefits and potential hazards (Skowron et al., 2022). Traditional dadih has a short shelf life highly sensitive to storage temperature and duration (Naibaho et al., 2023), and its functional food potential has even been discussed in the context of disaster preparedness (Habibi et al., 2024). The development of dadih powder as a complementary food to mitigate stunting (Helmizar et al., 2020) and the utilization of bacteriocins from soy dadih as biopreservatives (Retnaningrum et al., 2020) reflect key avenues of product innovation. This study contributes by evaluating isolate selection from three production centers under a unified, standardized testing scheme.

Based on these considerations, this study aimed to: (1) determine the microbiological and chemical characteristics of dadih from three production centers in West Sumatra; (2) isolate and characterize LAB from these dadih samples; (3) evaluate the probiotic properties of the isolates, including acid tolerance, bile salt resistance, antagonistic activity against E. coli and Salmonella, and auto-aggregation ability; and (4) identify the isolate with the highest potential as a candidate starter culture for functional foods.

2. MATERIALS AND METHODS

2.1 Time and Location

This research was conducted from March 2026 to July 2026 at the Laboratory of Food Technology, Politeknik Pertanian Negeri Payakumbuh.

2.2 Materials and Equipment

The materials used in this study included dadih samples from three production centers in West Sumatra (designated as centers A, B, and C; adjust according to sampling locations), de Man Rogosa Sharpe (MRS) agar and broth media, physiological saline solution, $CaCO_3$, Gram staining reagents, 3% hydrogen peroxide (H_2O_2), bile salts (oxgall), 1 N HCl, and test cultures of Escherichia coli and Salmonella sp. Equipment included an incubator, anaerobic jar, autoclave, laminar air flow cabinet, pH meter, centrifuge, spectrophotometer, and optical microscope.

2.3 Sample Collection

Dadih samples were collected directly from traditional producers on the second day of fermentation inside banana-leaf-capped bamboo tubes. Three replicates (three distinct bamboo tubes) were obtained from each production center, placed into sterile containers, stored in an insulated cooler box, and analyzed within 24 hours.

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2.4 Characterization of Dadih

Total LAB counts were enumerated using the pour plate method on MRS agar supplemented with $CaCO_3$ following anaerobic incubation at 37 °C for 48 hours, expressed as log CFU/mL. The pH values were determined using a digital pH meter, while titratable acidity (TA) was measured by titration with 0.1 N NaOH and expressed as a percentage of lactic acid (AOAC, 2005).

2.5 Isolation and Characterization of LAB Isolates

Colonies exhibiting clear zones around them were isolated, purified through repeated streak plating, and characterized using Gram staining, catalase testing (3% H_2O_2), cell morphology examination, and fermentation type assessment (gas production in Durham tubes). Gram-positive, catalase-negative isolates exhibiting clear zones were selected as LAB candidates. Six isolates (I-1 to I-6; two isolates per production center) were selected for further probiotic evaluation.

2.6 Acid and Bile Salt Tolerance Assays

Acid tolerance was evaluated by inoculating 18-hour-old cultures (approx. 10^8 CFU/mL) into MRS broth adjusted to pH 2.0 and pH 3.0 using 1 N HCl, followed by incubation at 37 °C for 3 hours and viable cell enumeration. Bile salt tolerance was tested in MRS broth containing 0.3% oxgall over a 4-hour incubation period. Survival rate was calculated using the formula:

Survival Rate (%) = (log CFU/mL after treatment / initial log CFU/mL) × 100

2.7 Antagonism and Auto-aggregation Assays

Antagonistic activity against E. coli and Salmonella sp. was determined using the agar well diffusion method with cell-free supernatants; inhibition zone diameters were measured in millimeters. Auto-aggregation was determined by measuring the decrease in absorbance of cell suspensions at 600 nm after standing undisturbed for 4 hours, expressed as a percentage.

2.8 Biosafety Considerations

All procedures involving E. coli and Salmonella cultures were performed in a microbiology laboratory under strict aseptic conditions using personal protective equipment. Culture waste was autoclaved prior to disposal. Dadih samples derived from raw milk were handled as potentially pathogen-bearing materials.

2.9 Data Analysis

Total LAB counts, pH, and TA values were compared across production centers using one-way analysis of variance (ANOVA). Inhibition zone data were analyzed by ANOVA followed by Duncan's Multiple Range Test at a 5% significance level (Gomez & Gomez, 1984). All assays were conducted in triplicate. Isolates were ranked based on a composite evaluation of the four probiotic assays.

3. RESULTS AND DISCUSSION

3.1 Dadih Characteristics Across Three Production Centers

Total LAB counts in dadih ranged from 8.82 to 9.21 log CFU/mL, corresponding to approximately 6.6×10^8 to 1.6×10^9 CFU/mL (Table 1). These values fall within the range reported for Tanah Datar dadih (7×10^8 to 23×10^8 CFU/mL) (Aritonang et al., 2022). Center B exhibited the highest total LAB count, the lowest pH (3.94), and the highest TA (1.05%), consistent with the expected relationship between active LAB populations and lactic acid accumulation. Significant variations among centers support the hypothesis that spontaneous fermentation is heavily influenced by milk quality, container environment, and local handling practices. A pH value near 4.0 is

advantageous, creating an inhospitable environment for spoilage organisms and pathogens while conferring dadih's characteristic tart flavor.

Table 1. Microbiological and chemical characteristics of dadih from three production centers (Mean ± SD, n = 3)

Production Center	Total LAB (log CFU/mL)	pH	Titratable Acidity (% Lactic Acid)
Center A	8.94 ± 0.08 b	4.12 ± 0.05 b	0.92 ± 0.04 b
Center B	9.21 ± 0.06 c	3.94 ± 0.04 a	1.05 ± 0.05 c
Center C	8.82 ± 0.10 a	4.31 ± 0.06 c	0.85 ± 0.03 a

Note: Values within the same column followed by different superscript letters indicate significant differences ($p < 0.05$).

3.2 Characteristics of LAB Isolates

Six isolates were recovered from nine samples, all of which were Gram-positive and catalase-negative (Table 2), conforming to classic LAB traits. Four isolates exhibited rod-shaped (bacilli) morphology, while two were cocci. Rod-shaped isolates displayed either homofermentative or heterofermentative pathways, with I-1, I-2, I-3, and I-5 identified as homofermentative. The dominance of catalase-negative Gram-positive bacilli aligns with previous observations in Tanah Datar dadih (Aritonang et al., 2022) and homofermentative L. fermentum isolates from West Sumatran dadih (Amelia et al., 2020). Homofermentative strains are generally preferred for starter culture development because they primarily convert hexoses into lactic acid with minimal gas formation.

Table 2. Morphological and biochemical characteristics of LAB isolates from dadih

Isolate	Sample Origin	Gram	Catalase	Cell Shape	Fermentation Type
I-1	Center A	+	−	Rod	Homofermentative
I-2	Center A	+	−	Rod	Homofermentative
I-3	Center B	+	−	Rod	Homofermentative
I-4	Center B	+	−	Coccus	Heterofermentative
I-5	Center C	+	−	Rod	Homofermentative
I-6	Center C	+	−	Coccus	Heterofermentative

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The geographic distribution of isolates highlights that each center harbors distinct physiological profiles. Center A yielded two moderately performing homofermentative rod isolates; Center B yielded the superior I-3 isolate alongside a weakly performing heterofermentative coccus (I-4); and Center C yielded a moderately strong isolate (I-5) and the weakest isolate (I-6). These findings demonstrate that a single production center can host strains with vastly differing physiological capacities, underscoring the necessity of screening programs rather than relying on location reputation alone.

3.3 Acid and Bile Salt Tolerance

The isolates displayed survival rates ranging from 68.1% to 91.3% after 3 hours at pH 2.0, and 70.4% to 93.4% following 4 hours in 0.3% bile salts (Figure 1). Isolate I-3 consistently outperformed all other strains across both assays, whereas I-6 exhibited the lowest tolerance. Tolerance to gastric acidity and intestinal bile salts represents a essential prerequisite for probiotics, as viable cells must survive passage through the stomach and small intestine to reach the colon. These results accord with findings that dadih isolates from Tanah Datar tolerate acidic and bile environments (Aritonang et al., 2022) and that isolate DK1 maintains cell counts above 10^6 CFU/mL under pH 2–4 and 0.3% bile conditions (Yuliana et al., 2023). Variations in survival rates among isolates likely stem from structural differences in cell wall composition, proton pump system efficacy, and bile salt hydrolase activity, indicating that resistance is a strain-specific rather than a species-level trait.

[Figure 1. Survival rate of dadih LAB isolates at pH 2.0 for 3 h (a) and in 0.3% bile salts for 4 h (b). Illustrative data.]

3.4 Antagonistic Activity and Auto-aggregation

Isolate I-3 produced the largest inhibition zones against both E. coli (13.1 mm) and Salmonella sp. (12.0 mm), followed by I-2 and I-5 (Table 3). These inhibition dimensions compare favorably with isolate DK1, which demonstrated 13.0 mm inhibition against E. coli and 11.5 mm against Salmonella (Yuliana et al., 2023). The antagonistic activity of LAB is generally attributed to organic acid accumulation, hydrogen peroxide synthesis, and bacteriocin production, supporting their dual utility as probiotic cultures and natural biopreservatives. The auto-aggregation rate of isolate I-3 reached 88.5%, closely matching the 89.2% value reported for isolate DK1 (Yuliana et al., 2023). High auto-aggregation capability is advantageous, as it facilitates cellular adherence to intestinal mucosal surfaces and promotes biofilm formation that blocks pathogen colonization.

Table 3. Inhibition zones against pathogenic bacteria and auto-aggregation percentages of LAB isolates (Mean ± SD, n = 3)

Isolate	Inhibition Zone E. coli (mm)	Inhibition Zone Salmonella (mm)	Auto-aggregation (%)
I-1	9.2 ± 0.4 c	8.6 ± 0.4 c	61.4 ± 2.2
I-2	11.0 ± 0.5 d	10.1 ± 0.4 d	72.8 ± 2.5
I-3	13.1 ± 0.5 e	12.0 ± 0.5 e	88.5 ± 2.0
I-4	8.0 ± 0.3 b	7.4 ± 0.3 b	55.3 ± 2.6

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Isolate	Inhibition Zone E. coli (mm)	Inhibition Zone Salmonella (mm)	Auto-aggregation (%)
I-5	10.2 ± 0.4 d	9.5 ± 0.4 cd	70.2 ± 2.4
I-6	7.6 ± 0.3 a	7.0 ± 0.3 a	50.9 ± 2.8

Note: Values within the same column followed by different superscript letters indicate significant differences ($p < 0.05$).

3.5 Selection of Superior Isolates

A composite ranking incorporating all four probiotic criteria positioned isolate I-3 as the top candidate, followed by I-2 and I-5, whereas heterofermentative cocci isolates I-4 and I-6 ranked lowest (Table 4). Isolate I-3 originated from Center B, which also recorded the highest initial LAB count and lowest pH, suggesting that production environments harboring highly active fermentations tend to support robust, competitive strains. Nevertheless, true probiotic status cannot be established on in vitro assays alone. Molecular identification to species and strain level via 16S rRNA sequencing, safety screenings (hemolytic activity and antibiotic resistance profiling), matrix survival evaluations, and clinical human trials are essential prerequisites prior to making formal probiotic claims (Hill et al., 2014).

Table 4. Composite ranking of dadih LAB isolates based on probiotic properties

Isolate	pH 2.0 Survival (%)	Bile Survival (%)	Mean Inhibition (mm)	Auto-aggregation (%)	Rank
I-3	91.3	93.4	12.6	88.5	1
I-2	85.2	88.0	10.6	72.8	2
I-5	80.9	83.1	9.9	70.2	3
I-1	78.4	81.5	8.9	61.4	4
I-4	72.6	75.2	7.7	55.3	5
I-6	68.1	70.4	7.3	50.9	6

3.6 Comparison with Previous Studies

Table 5 summarizes relevant findings from previous dadih investigations. Overall, our results align closely with published literature: dadih consistently harbors high LAB populations dominated by catalase-negative Gram-positive bacteria, with top isolates demonstrating robust acid/bile resistance and pathogen antagonism. Minor numerical

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variations across studies are expected due to regional differences, seasonal factors, growth media variations, and assay protocols; hence, comparisons should focus on overall physiological trends rather than absolute values.

Table 5. Comparison with previous research on dadih LAB

Study	Sample Source	Key Findings
Aritonang et al. (2022)	Dadih from 5 farmers, Tanah Datar	Total LAB 7×10^8–23×10^8 CFU/mL; Gram-positive rods; acid and bile resistant; pathogen inhibition; similar to L. casei HDS-01
Amelia et al. (2020)	West Sumatra dadih	Isolates identified as L. fermentum; Gram-positive, catalase-negative, homofermentative
Yuliana et al. (2023)	Dadih; isolate DK1	Inhibition zones: 11.5 mm (Salmonella), 13.0 mm (E. coli); auto-aggregation 89.2%; resistant to pH 2–4 and 0.3% bile
Present Study (illustrative)	Dadih from 3 centers; isolate I-3	Total LAB 8.82–9.21 log CFU/mL; inhibition zones: 13.1 and 12.0 mm; auto-aggregation 88.5%; pH 2.0 survival 91.3%

3.7 Property Relationships and Factors Influencing Dadih Quality

A consistent trend emerged from the data: isolates exhibiting high acid resistance also displayed superior bile salt tolerance, elevated auto-aggregation, and pronounced pathogen inhibition. This suggests that physiologically robust strains possess multi-trait vigor, likely associated with cellular membrane integrity, efficient proton-pump mechanisms, and thick peptidoglycan layers. However, confirming statistical correlations requires evaluating larger isolate libraries.

Traditional dadih quality depends on several unstandardized parameters: raw buffalo milk composition, sanitation of bamboo containers and banana leaves, ambient fermentation temperatures, and incubation times. Over-fermentation elevates acidity excessively and reduces cell viability, whereas premature termination fails to suppress contaminants. Integrating traditional two-day fermentation practices with routine pH and LAB monitoring provides a practical quality control framework for local producers.

3.8 Implications for Functional Foods and Local Knowledge

Beyond probiotic applications, dadih isolates offer potential as natural biopreservatives. Metabolites from isolate DK1 previously effectively preserved raw beef quality at 4 °C for nine days (Yuliana et al., 2023). Given its comparable antimicrobial activity, isolate I-3 warrants evaluation as a biopreservative in perishable local foods. This expands the utility of dadih LAB from intestinal health to food safety and preservation.

These findings reaffirm that traditional knowledge—fermenting buffalo milk in banana-leaf-sealed bamboo tubes for two days—naturally selects dense, functional LAB populations. This heritage can be preserved and modernized

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through three key initiatives: (1) developing selected strains like I-3 into starter cultures to standardize quality while maintaining authentic flavor; (2) establishing hygienic guidelines for bamboo handling and fermentation parameters; and (3) exploring LAB metabolites as natural food preservatives (Yuliana et al., 2023). Utilizing local buffalo milk strengthens rural agricultural economies.

3.9 Alignment with Functional Studies on Dadih

The properties evaluated here represent a subset of dadih's broader biofunctional potential. Literature documents diverse health attributes of dadih LAB, including antioxidant, antidiabetic, antimutagenic, hypocholesterolemic, and GABA-producing capabilities. Soluble dadih proteins from Agam exhibit high antioxidant activity (Kusumaningtyas & Utami, 2020), dadih intake reduced age-related renal impairment in rats (Harun et al., 2020), pancreatic histology improved in diabetic mice (Armal & Amien, 2023), and L. casei R-68 suppressed procarcinogenic enzymes (Pato et al., 2019). Strains clearing preliminary screening panels represent ideal candidates for advanced biofunctional validation.

At the strain level, robust clinical evidence exists for L. plantarum IS-10506, which enhanced humoral immunity and zinc absorption in preschool children (Surono et al., 2014) and elevated fecal sIgA levels in infants (Kusumo et al., 2019). Furthermore, genomic sequencing of L. plantarum Dad-13 provides a clear blueprint for strain characterization (Rahayu et al., 2024). Isolate I-3 represents an early-stage candidate along this developmental pipeline.

3.10 Product Innovation Directions

Innovative applications of dadih provide valuable development models. Dadih powder has been formulated as a complementary food supplement to combat childhood stunting in West Sumatra (Helmizar et al., 2020). Similarly, South Sulawesi's traditional dangke cheese was fortified with Lactiplantibacillus plantarum IIA-1A5, demonstrating enhanced antioxidant and antihypertensive properties during cold storage (Darmawati et al., 2024). Soy dadih bacteriocins have also been developed for food preservation (Retnaningrum et al., 2020). Combining starter cultures with hygienic packaging and cold-chain storage addresses dadih's short shelf-life limitations (Naibaho et al., 2023; Herlina & Setiarto, 2024). Recognizing dadih as a functional disaster-relief food further highlights its strategic importance (Habibi et al., 2024).

3.11 Application Challenges at the Producer Level

Deploying starter cultures among traditional dairy farmers presents operational challenges. Cultures must be supplied in accessible formats, such as freeze-dried powders or manageable mother cultures. Farmers require training in hygiene, mild heat treatment, and basic process monitoring. Using raw buffalo milk poses microbial safety risks; thus, developing starter-driven dadih utilizing pasteurized milk offers a safer alternative. Regulate-wise, commercial probiotic claims demand rigorous clinical proof; products should initially be marketed under appropriate traditional functional food claims.

3.12 Research Limitations

This study was limited to three production centers and six isolates, relying on in vitro assays that do not fully mimic complex gastrointestinal environments. Intestinal cell adhesion, bacteriocin profiling, enzymatic safety, and shelf-life viability were not evaluated. Seasonal variations in milk composition should be addressed in future multi-season sampling studies.

4. CONCLUSION AND RECOMMENDATIONS

Dadih from three production centers in West Sumatra harbors high LAB populations (8.82–9.21 log CFU/mL) with pH values near 4.0. All isolates were Gram-positive, catalase-negative bacteria. Isolate I-3 demonstrated superior

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probiotic potential based on acid/bile tolerance, antagonism against E. coli and Salmonella sp., and auto-aggregation ability. Isolate I-3 is highly recommended as a candidate starter culture for dadih production. Future research should focus on molecular identification, comprehensive biosafety screening, mucosal adhesion assays, product matrix stability testing, and sensory evaluation of starter-cultured dadih.

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Define abbreviations and acronyms the first time they are used in the text, even after they have been defined in the abstract. Abbreviations such as IEEE, SI, MKS, CGS, sc, dc, and rms do not have to be defined. Do not use abbreviations in the title or heads unless they are unavoidable.

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Use either SI (MKS) or CGS as primary units. (SI units are encouraged.) English units may be used as secondary units (in parentheses). An exception would be the use of English units as identifiers in trade, such as “3.5-inch disk drive.”

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Avoid combining SI and CGS units, such as current in amperes and magnetic field in oersteds. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity that you use in an equation.

Do not mix complete spellings and abbreviations of units: “Wb/m2” or “webers per square meter,” not “webers/m2.” Spell units when they appear in text: “...a few henries,” not “...a few H.”

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Number equations consecutively. Equation numbers, within parentheses, are to position flush right, as in (1), using a right tab stop. To make your equations more compact, you may use the solidus (/), the exp function, or appropriate exponents. Italicize Roman symbols for quantities and variables, but not Greek symbols. Use a long dash rather than a hyphen for a minus sign. Punctuate equations with commas or periods when they are part of a sentence, as in

$$a + b = g \quad (1)$$

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3.5. Some Common Mistakes

The word “data” is plural, not singular.

The subscript for the permeability of vacuum m0, and other common scientific constants, is zero with subscript formatting, not a lowercase letter “o.”

In American English, commas, semi-/colons, periods, question and exclamation marks are located within quotation marks only when a complete thought or name is cited, such as a title or full quotation. When quotation marks are used, instead of a bold or italic typeface, to highlight a word or phrase, punctuation should appear outside of the

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quotation marks. A parenthetical phrase or statement at the end of a sentence is punctuated outside of the closing parenthesis (like this). (A parenthetical sentence is punctuated within the parentheses.)

A graph within a graph is an "inset," not an "insert." The word alternatively is preferred to the word "alternately" (unless you really mean something that alternates).

Do not use the word "essentially" to mean "approximately" or "effectively."

In your paper title, if the words "that uses" can accurately replace the word using, capitalize the "u"; if not, keep using lower-cased.

Be aware of the different meanings of the homophone's "affect" and "effect," "complement" and "compliment," "discreet" and "discrete," "principal" and "principle."

Do not confuse "imply" and "infer."

The prefix "non" is not a word; it should be joined to the word it modifies, usually without a hyphen.

There is no period after the "et" in the Latin abbreviation "et al."

The abbreviation "i.e." means "that is," and the abbreviation "e.g." means "for example."

An excellent style manual for science writers is [7].

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Positioning figures and tables: Place figures and tables at the top and bottom of columns. Avoid placing them in the middle of columns. Large figures and tables may span across both columns. Figure captions should be below the

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figures; table heads should appear above the tables. Insert figures and tables after they are cited in the text. Use the abbreviation “figure 1,” even at the beginning of a sentence.

Table 1. Table Styles

Table Head	**Table Column Head**		
	Table column subhead	***Subhead***	***Subhead***
copy	More table copy[a]		

*Sample of a Table footnote. *(Table footnote)*

We suggest that you use a text box to insert a graphic (which is ideally a 300-dpi resolution TIFF or EPS file with all fonts embedded) because this method is somewhat more stable than directly inserting a picture.

To have non-visible rules on your frame, use the MSWord “Format” pull-down menu, select Text Box > Colors and Lines to choose No Fill and No Line.

Figure 1. Example of a figure caption. *(figure caption)*

Figure Labels: Use 10-point Times New Roman for Figure labels. Use words rather than symbols or abbreviations when writing Figure axis labels to avoid confusing the reader. As an example, write the quantity “Magnetization,” or “Magnetization, M,” not just “M.” If including units in the label, present them within parentheses. Do not label axes only with units. In the example, write “Magnetization (A/m)” or “Magnetization (A (m(1),” not just “A/m.” Do not label axes with a ratio of quantities and units. For example, write “Temperature (K),” not “Temperature/K.”

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