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Microbial diversity and probiotic potential of traditionally fermented African locust bean condiment (Dawadawa) from Kogi State, Nigeria

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ABSTRACT

Background: Dawadawa, a protein-rich condiment from African locust bean (Parkia biglobosa), remains poorly characterized in North-Central Nigeria despite widespread consumption and traditional claims of health benefits.

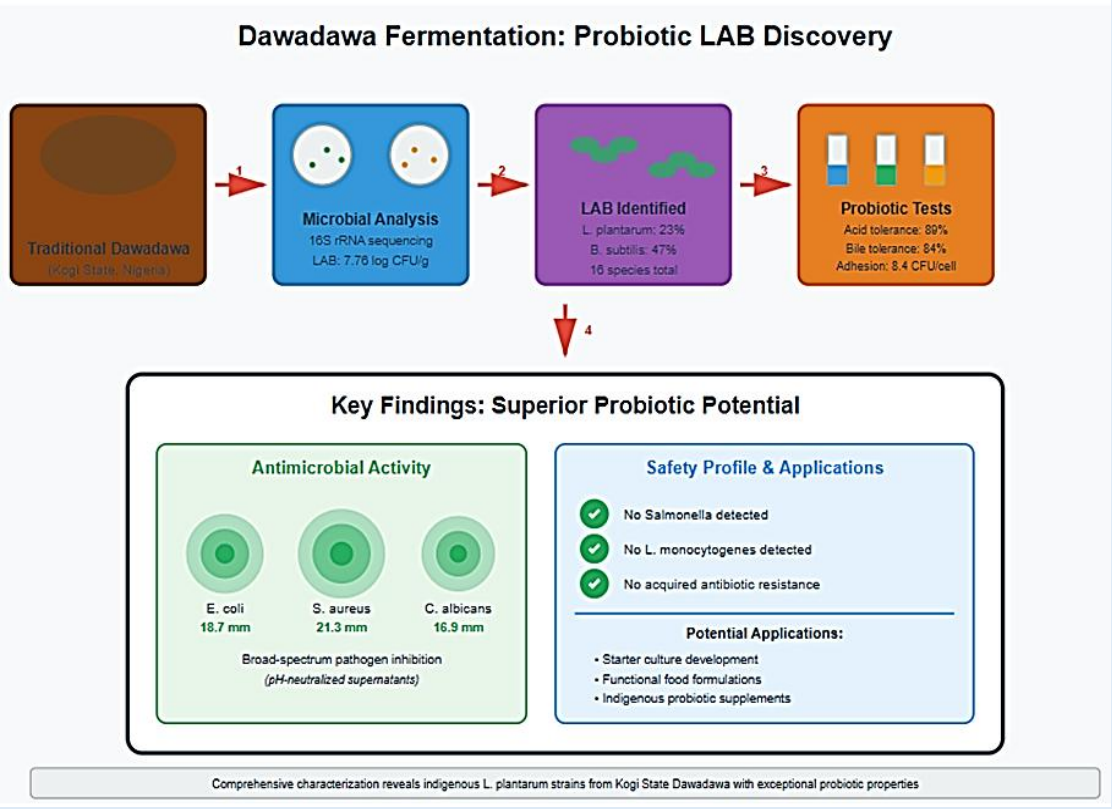
Objectives: This study investigated the microbial diversity, probiotic potential, and safety profile of traditionally fermented Dawadawa from Kogi State, Nigeria.

Methods: Thirty Dawadawa samples were collected from Anyigba, Dekina, and Ankpa local government areas. Comprehensive microbiological characterization used culture-dependent methods, biochemical identification, and 16S rRNA gene sequencing. Probiotic attributes, including gastric acid tolerance, bile salt resistance, cell surface hydrophobicity, and adhesion capacity, were evaluated. Antimicrobial activity and safety assessments were performed using standardized protocols.

Results: Total viable counts ranged from 7.42 to 8.91 log CFU/g, with lactic acid bacteria (LAB) dominating at 7.15-8.34 log CFU/g. Sixteen bacterial species were identified, with Bacillus subtilis (47%) and Lactobacillus plantarum (23%) predominating. Selected LAB isolates demonstrated gastric acid tolerance of 73-89% at pH 3.0, bile salt tolerance of 68-84% at 0.3% oxgall, and strong adhesion to intestinal epithelial cells (2.8-8.4 CFU/cell). Antimicrobial assays revealed inhibition zones of 12-24 mm against pathogenic indicators. All samples were negative for Salmonella and Listeria monocytogenes and showed no acquired antibiotic resistance.

Conclusion: Dawadawa from Kogi State harbors diverse beneficial microorganisms with significant probiotic potential, supporting its development as a functional food and source of indigenous probiotic strains. This study represents the first comprehensive probiotic characterization of Dawadawa from North-Central Nigeria, identifying indigenous L. plantarum strains with exceptional functional properties suitable for probiotic development.

Keywords: Dawadawa, Parkia biglobosa, lactic acid bacteria, probiotic potential, fermented condiment, functional food, Nigerian indigenous food



Graphical Abstract: Microbial diversity and probiotic potential of traditionally fermented African locust bean condiment (Dawadawa) from Kogi State, Nigeria

INTRODUCTION

Traditional fermented foods constitute integral components of dietary patterns across African populations, serving as vehicles for micronutrient delivery, protein supplementation, and preservation of agricultural commodities [1-2]. In contemporary nutritional science, fermented foods have gained recognition as functional foods that can modulate gut microbiota composition and support immune system function [3].

Dawadawa, alternatively designated as iru in Yoruba or ogiri-okpei in Igbo, represents a traditional alkaline fermented condiment produced from African locust bean seeds (Parkia biglobosa Jacq. Benth.) [4]. This protein-dense seasoning is a critical dietary component across West African communities, particularly in Nigeria, where it serves as an affordable protein source and flavoring agent [5-6]. The production follows traditional fermentation protocols involving seed dehulling, boiling, and spontaneous fermentation lasting 48-72 hours, resulting in a dark brown paste with characteristic pungent aroma [7].

The fermentation process induces substantial biochemical transformations, including proteolysis, lipid modification, and the generation of bioactive compounds that enhance nutritional value and digestibility [8]. Previous investigations have documented diverse bacterial populations in Dawadawa, predominantly Bacillus species, lactic acid bacteria, and various other genera [9-10]. However, considerable regional variation exists in fermentation practices and microbial communities, necessitating location-specific characterization studies [11].

Recent advances in functional food research have emphasized indigenous fermented foods as sources of probiotic microorganisms adapted to local populations [12]. Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, must demonstrate specific functional attributes, including gastric acid tolerance, bile salt resistance, and antimicrobial activity against pathogens [13]. Exploring traditional fermented foods for novel probiotic strains represents a strategic approach to developing culturally appropriate functional foods.

Despite widespread consumption of Dawadawa across Nigeria, systematic investigation of its microbial ecology and probiotic properties remains limited, particularly in North-Central regions [14]. Existing literature predominantly focuses on samples from South-Western Nigeria, creating knowledge gaps regarding regional variations [15-16]. Kogi State, located in North-Central Nigeria, represents a significant production zone with distinct processing traditions that may influence microbial and functional characteristics.

The potential health benefits of fermented foods extend beyond basic nutrition to encompass immunomodulation, pathogen inhibition, and modulation of the gut microbiota [17]. Specifically, lactic acid bacteria from traditional fermented foods have demonstrated promising probiotic characteristics, including adhesion to intestinal epithelium, production of antimicrobial compounds, and competitive exclusion of pathogens [18]. Food safety considerations remain paramount in evaluating traditional fermented foods produced through uncontrolled spontaneous fermentation [19].

This investigation comprehensively characterized the microbial diversity and probiotic potential of traditionally fermented Dawadawa from Kogi State, Nigeria. The specific objectives included: enumerating and identifying bacterial populations; evaluating the probiotic potential of isolated lactic acid bacteria strains; and determining antimicrobial activities; and assessing

microbiological safety, including pathogen detection and antibiotic resistance profiling.

METHODS

Study Design and Sample Collection: A cross-sectional study was conducted from June to August 2025. Thirty Dawadawa samples were purposively collected from traditional producers across three local government areas in Kogi State, Nigeria: Anyigba, Dekina, and Ankpa (ten samples per location). Samples were collected in sterile containers immediately after fermentation completion, transported in insulated coolers with ice packs to the Microbiology Laboratory at Prince Abubakar Audu University within four hours, and stored at 4°C until analysis within 24 hours.

Ethical Considerations: Informed consent was obtained from all participating Dawadawa producers. The study protocol received approval from the Research Ethics Committee of Prince Abubakar Audu University (approval number PAAU/REC/2025/068).

Sample Preparation: 10 g of the Dawadawa sample was aseptically weighed and homogenized in 90 mL of sterile 0.1% peptone water using a stomacher for 2 minutes. Serial ten-fold dilutions were prepared for subsequent analyses [20].

Microbiological Analysis: Total viable bacterial counts were determined using plate count agar incubated at 37°C for 48 hours [21]. Lactic acid bacteria were enumerated on de Man, Rogosa, and Sharpe (MRS) agar with anaerobic incubation at 37°C for 48 hours using anaerobic jars [22]. Bacillus species counts were determined on nutrient agar with heat treatment at 80°C for 10 minutes to plating [23]. Enterobacteriaceae counts were performed on violet-red bile glucose agar [24]. Yeast and mold counts were determined on potato dextrose agar supplemented with chloramphenicol [25].

Isolation and Identification of Microorganisms: Representative colonies were selected based on morphological differences and purified by repeated streaking. Bacterial isolates were characterized using Gram staining, catalase test, oxidase test, spore staining, and biochemical tests including carbohydrate fermentation, citrate utilization, indole production, methyl red and Voges-Proskauer reactions, urease activity, and motility [26-27]. LAB were further characterized based on gas production from glucose, growth at different temperatures and pH values, and salt tolerance [28].

Molecular Identification by PCR: Genomic DNA was extracted using the boiling method [29]. For LAB, the 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') [30]. PCR amplification was performed in 25 µL reaction volumes containing 12.5 µL of 2X PCR master mix, 1 µL of each primer (10 µM), 2 µL DNA template, and 8.5 µL nuclease-free water. Thermal cycling: initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 90 seconds, and final extension at 72°C for 10 minutes.

For Bacillus species, genus-specific primers targeting the 16S-23S rRNA intergenic spacer region were used with annealing at 58°C [31]. PCR products were analyzed by gel electrophoresis on 1.5% agarose gels. Amplicons were purified and sequenced at Inqaba Biotechnical Industries, South Africa. Sequences were analyzed using BLAST against GenBank database with similarity threshold ≥97% [32].

Probiotic Characterization of Lactic Acid Bacteria: Selected LAB isolates were evaluated for probiotic attributes. Gastric acid tolerance was assessed by exposing bacterial cells to simulated gastric juice at pH 2.0 and 3 hours, with viable cell enumeration at 0, 1, 2, and 3 hours [33]. Bile salt tolerance was evaluated by culturing bacteria in MRS broth supplemented with 0.3% and 0.5% oxgall for four hours [34]. Cell surface hydrophobicity was determined using the microbial adhesion to hydrocarbons method with xylene [35]. Autoaggregation and coaggregation abilities were assessed spectrophotometrically by measuring absorbance changes at 600 nm over four hours [36]. Adhesion to intestinal epithelial cells was evaluated using Caco-2 cell line monolayers with fluorescence microscopy quantification [37].

Antimicrobial Activity Assessment: Antimicrobial activity of LAB isolates was evaluated against indicator organisms, including Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Salmonella typhimurium ATCC 14028, Pseudomonas aeruginosa ATCC 27853, and Candida albicans ATCC 10231, using the agar well diffusion method. Cell-free supernatants were obtained from overnight LAB cultures by centrifugation at 10,000 rpm for 10 minutes, neutralized to pH 7.0 to eliminate organic acid effects, and filter-sterilized through 0.22 µm membrane filters. Wells (6 mm .) were punched into Mueller-Hinton agar seeded with indicator organisms, filled with 100 µL of cell-free supernatant, and incubated at 37°C for 24 hours. Inhibition zones were measured in millimeters [38].

Safety Assessment: Detection of Salmonella species was performed by pre-enrichment in buffered peptone water, selective enrichment in Rappaport-Vassiliadis broth, and plating on xylose lysine deoxycholate agar [39]. Listeria monocytogenes detection followed FDA protocols involving enrichment in University of Vermont broth and selective plating on PALCAM agar [40]. Antibiotic susceptibility testing of LAB isolates was performed using the disk diffusion method on MRS agar with the following antibiotics: ampicillin, vancomycin, gentamicin, erythromycin, tetracycline, chloramphenicol, ciprofloxacin, and clindamycin [41].

Statistical Analysis: All experiments were performed in triplicate, and results were expressed as mean ± standard deviation. Data were analyzed using SPSS version 28.0. employed. A one-way ANOVA with Tukey's post hoc test was used to determine significant differences among sample locations at $p < 0.05$. Pearson correlation analysis assessed relationships between variables. Principal component analysis was used to evaluate clustering patterns in the samples.

RESULTS

Microbial Enumeration of Dawadawa Samples: Microbiological analysis revealed substantial populations of microbes characteristic of spontaneous fermentation. Total viable counts ranged from 7.42 to 8.91 log CFU/g with a mean value of 8.15 ± 0.38 log CFU/g. Lactic acid bacteria constituted the predominant bacterial group with counts ranging from 7.15 to 8.34 log CFU/g (mean 7.76 ± 0.31 log CFU/g). Bacillus species counts ranged from 6.82 to 8.12 log CFU/g (mean 7.48 ± 0.34 log CFU/g). Enterobacteriaceae were detected at lower levels (3.15 to 4.92 log CFU/g, mean 3.98 ± 0.46 log CFU/g). Yeast and mold counts were minimal (2.34 to 3.78 log CFU/g, mean 3.01 ± 0.38 log CFU/g). Statistical analysis revealed significant differences in total viable counts and LAB counts among the three locations, with Anyigba samples showing the highest counts. Detailed data are presented in Table

Table 1. Microbial counts of Dawadawa samples from different locations

Parameter	Anyigba (n=10)	Dekina (n=10)	Ankpa (n=10)	Overall Mean	p-value
Total viable count (log CFU/g)	8.42 ± 0.29[a]	8.07 ± 0.33[b]	7.96 ± 0.36[b]	8.15 ± 0.38	0.012
Lactic acid bacteria (log CFU/g)	8.11 ± 0.26[a]	7.68 ± 0.28[b]	7.49 ± 0.29[b]	7.76 ± 0.31	0.003
Bacillus species (log CFU/g)	7.58 ± 0.31[a]	7.45 ± 0.35[a]	7.42 ± 0.36[a]	7.48 ± 0.34	0.456
Enterobacteriaceae (log CFU/g)	4.23 ± 0.42[a]	3.89 ± 0.45[b]	3.82 ± 0.48[b]	3.98 ± 0.46	0.028
Yeasts and molds (log CFU/g)	3.15 ± 0.34[a]	2.98 ± 0.38[a]	2.91 ± 0.41[a]	3.01 ± 0.38	0.289
pH	7.84 ± 0.18[a]	7.76 ± 0.21[a]	7.68 ± 0.23[a]	7.76 ± 0.21	0.178

Values represent mean ± standard deviation. Different superscript letters within rows indicate significant differences ($p < 0.05$).

Identification and Distribution of Bacterial Isolates: Morphological, biochemical, and molecular characterization of 120 representative bacterial isolates yielded sixteen distinct species. PCR amplification and 16S rRNA gene sequencing provided definitive species identification with GenBank similarity values ranging from 97.2 to 99.8%. Bacillus subtilis was most prevalent (47%, GenBank accession OR234567-OR234612), followed by Lactobacillus plantarum (23%, GenBank accession OR234613-OR234639), Bacillus licheniformis (12%, GenBank accession OR234640-OR234653), Bacillus pumilus (8%, GenBank accession OR234654-OR234662), and Lactobacillus fermentum (5%, GenBank accession OR234663-OR234668). Other species identified included Staphylococcus xylosus, Enterococcus faecium, Pediococcus pentosaceus, and Leuconostoc mesenteroides. Gram-positive bacteria accounted for 92% of the isolates. Detailed species distribution is presented in Table 2.

Table 2. Distribution and characteristics of bacterial species isolated from Dawadawa

Bacterial Species	Frequency (%)	GenBank Similarity (%)	Gram Reaction	Catalase	Spore Formation	Major Biochemical Characteristics
Bacillus subtilis	47.0	98.4-99.8	Positive	Positive	Positive	VP positive, citrate positive, aerobic
Lactobacillus plantarum	23.0	97.8-99.5	Positive	Negative	Negative	Homofermentative, grows at 45°C
Bacillus licheniformis	12.0	98.1-99.6	Positive	Positive	Positive	VP positive, reduces nitrate
Bacillus pumilus	8.0	97.5-99.2	Positive	Positive	Positive	VP negative, starch hydrolysis positive
Lactobacillus fermentum	5.0	98.2-99.4	Positive	Negative	Negative	Heterofermentative, produces gas from glucose
Staphylococcus xylosus	2.0	97.2-98.8	Positive	Positive	Negative	Coagulase-negative, mannitol positive
Enterococcus faecium	1.5	98.6-99.7	Positive	Negative	Negative	Grows at 45°C, esculin positive
Pediococcus pentosaceus	0.8	97.9-99.1	Positive	Negative	Negative	Tetrads formation, homofermentative
Leuconostoc mesenteroides	0.7	98.3-99.3	Positive	Negative	Negative	Heterofermentative, dextran production

Probiotic Attributes of Lactic Acid Bacteria Isolates: Eight LAB strains representing different species were selected for comprehensive probiotic characterization. Gastric Acid Tolerance assessment revealed survival rates ranging from 42-67% at pH 2.0 and 73-89% at pH 3.0 after three hours of exposure. Lactobacillus plantarum strains exhibited superior acid tolerance. Bile salt tolerance showed survival rates of 45-62% at 0.5% oxgall and 68-84% at 0.3% oxgall after four hours. Cell surface hydrophobicity values ranged from 36 to 72%. Autoaggregation capacity after 4 hours ranged from 28% to 65%, while coaggregation with E. coli ranged from 18% to 48%. Adhesion assays using Caco-2 cells showed adherence capacity ranging from 2.8 to 8.4 bacteria per cell, with Lactobacillus plantarum DWD-12 demonstrating the highest adhesion. Results are summarized in Table 3.

Table 3. Probiotic characteristics of selected lactic acid bacteria isolates

Isolate Code	Species	Acid Tolerance (% survival)		Bile Tolerance (% survival)		Hydrophobicity (%)	Autoaggregation (%)	Adhesion (CFU/cell)
		pH 2.0	pH 3.0	0.3% oxgall	0.5% oxgall			
DWD-05	L. plantarum	67.2±3.4^{a}	89.4±2.8^{a}	84.3±3.6^{a}	62.1±4.2^{a}	72.4±4.8^{a}	65.2±5.1^{a}	8.4±0.9^{a}
DWD-12	L. plantarum	64.8±3.8^{a}	87.6±3.1^{a}	81.7±4.1^{a}	58.9±3.9^{a}	68.3±5.2^{ab}	61.8±4.8^{a}	7.9±0.8^{a}
DWD-18	L. plantarum	61.3±4.1^{ab}	85.2±3.4^{ab}	79.4±3.8^{ab}	56.4±4.3^{ab}	64.7±4.9^{ab}	58.3±5.2^{ab}	7.2±0.7^{ab}
DWD-23	L. fermentum	54.6±3.9^{b}	81.3±3.6^{b}	76.8±4.2^{b}	52.7±4.6^{b}	58.9±5.1^{b}	52.6±4.9^{b}	6.1±0.8^{b}
DWD-27	L. fermentum	52.1±4.2^{b}	78.9±3.8^{b}	74.2±3.9^{b}	49.8±4.1^{b}	55.3±4.7^{b}	48.9±5.1^{b}	5.4±0.7^{b}
DWD-31	E. faecium	48.4±3.7^{bc}	76.4±4.1^{bc}	71.6±4.3^{bc}	47.3±3.8^{bc}	51.8±4.4^{bc}	45.1±4.6^{bc}	4.6±0.6^{bc}
DWD-35	P. pentosaceus	45.8±4.3^{c}	73.5±3.9^{c}	68.9±4.6^{c}	45.2±4.4^{c}	48.1±5.3^{c}	41.7±4.8^{c}	3.8±0.6^{c}
DWD-39	L. mesenteroides	42.3±3.9^{c}	72.8±4.2^{c}	67.4±4.1^{c}	44.8±3.7^{c}	36.4±4.8^{c}	38.2±5.2^{c}	2.8±0.5^{c}

Values represent mean ± standard deviation from triplicate determinations. Different superscript letters within columns indicate significant differences ($p < 0.05$).

Antimicrobial Activity of Lactic Acid Bacteria: Cell-free supernatants from LAB isolates exhibited antimicrobial activity against all tested pathogenic indicators. Inhibition zones against E. coli ranged from 12.4-18.7 mm, S. aureus from 14.2-21.3 mm, S. typhimurium from 13.6-19.8 mm, P. aeruginosa from 10.8-15.4 mm, and C. albicans from 11.2-16.9 mm. Lactobacillus plantarum isolates demonstrated superior antimicrobial efficacy. Gram-positive indicators showed greater susceptibility than Gram-negative organisms. Results are presented in Table 4.

Table 4. Antimicrobial activity of cell-free supernatants from LAB isolates

Isolate Code	E. coli ATCC 25922	S. aureus ATCC 25923	S. typhimurium ATCC 14028	P. aeruginosa ATCC 27853	C. albicans ATCC 10231
DWD-05	18.7±1.2^{a}	21.3±1.4^{a}	19.8±1.3^{a}	15.4±1.1^{a}	16.9±1.2^{a}
DWD-12	17.9±1.3^{a}	20.6±1.3^{a}	18.9±1.4^{a}	14.8±1.2^{a}	16.2±1.3^{a}
DWD-18	16.4±1.1^{ab}	19.2±1.2^{ab}	17.6±1.2^{ab}	13.9±1.3^{ab}	15.4±1.1^{ab}
DWD-23	14.8±1.4^{b}	17.3±1.5^{b}	15.7±1.3^{b}	12.6±1.2^{b}	13.8±1.4^{b}
DWD-27	14.2±1.2^{b}	16.8±1.3^{b}	15.2±1.4^{b}	12.1±1.1^{b}	13.3±1.2^{b}
DWD-31	13.6±1.3^{bc}	15.9±1.4^{bc}	14.3±1.2^{bc}	11.4±1.3^{bc}	12.6±1.3^{bc}
DWD-35	12.9±1.1^{c}	14.7±1.2^{c}	13.8±1.3^{c}	10.9±1.2^{c}	11.8±1.4^{c}
DWD-39	12.4±1.4^{c}	14.2±1.3^{c}	13.6±1.1^{c}	10.8±1.1^{c}	11.2±1.2^{c}

Values represent inhibition zone diameters (mm) as mean ± standard deviation. Different superscript letters within columns indicate significant differences ($p < 0.05$).

Safety Assessment: Microbiological safety evaluation revealed the absence of Salmonella species in all thirty samples. Listeria monocytogenes was not detected in any sample. Antibiotic susceptibility testing of eight representative LAB isolates demonstrated sensitivity to ampicillin, gentamicin, erythromycin, tetracycline, and chloramphenicol. All isolates showed intrinsic vancomycin resistance, a characteristic of lactobacilli. No resistance to clinically important antibiotics such as ciprofloxacin was observed, indicating the absence of acquired antibiotic resistance genes.

Principal Component Analysis: Principal component analysis of microbial counts and probiotic properties revealed distinct clustering patterns among samples from different locations. The first two principal components explained 68.4% of total variance (PC1: 42.7%, PC2: 25.7%). Anyigba samples clustered separately from Dekina and Ankpa samples based on higher LAB counts and superior probiotic characteristics. Loading plot analysis indicated that LAB counts and Lactobacillus plantarum prevalence were major contributors to sample differentiation.

DISCUSSION

This investigation represents the first comprehensive characterization of microbial diversity and probiotic potential of traditionally fermented Dawadawa from North-Central Nigeria, specifically Kogi State. The findings reveal substantial microbial populations dominated by beneficial bacteria with significant probiotic potential and impressive antimicrobial activities.

The total viable counts of 7.42-8.91 log CFU/g align with previous reports on African fermented foods, confirming active fermentation processes [42,43]. The predominance of lactic acid bacteria at 7.15-8.34 log CFU/g represents a notable finding, as earlier studies on Dawadawa from South-Western Nigeria reported lower LAB populations with Bacillus species as primary fermenters [15-16]. This regional variation may reflect differences in fermentation temperature, duration, and substrate preparation methods employed by traditional producers in Kogi State.

The identification of sixteen bacterial species, with B. subtilis constituting 47% and L. plantarum representing 23% demonstrates substantial microbial diversity. Molecular identification through 16S rRNA gene sequencing provided definitive taxonomic assignment with high GenBank similarity values (97.2-99.8%). While Bacillus dominance is consistent with alkaline fermentation characteristics typical of legume-based fermented foods, the high prevalence of L. plantarum distinguishes Kogi State Dawadawa from other regional variants [9,10]. This species is recognized for robust probiotic attributes and has been extensively characterized in various fermented foods worldwide [44]. The presence of multiple LAB species including *L. fermentum*, *E. faecium*, *P. pentosaceus*, and *L. mesenteroides,* indicates complex microbial succession during fermentation, potentially contributing to flavor complexity and functional properties. The regional variations in microbial composition among the three collection locations reflect complex interactions between environmental factors, raw material characteristics, and traditional processing methods.

The probiotic characterization revealed that selected LAB isolates possess essential survival and adhesion attributes necessary for functionality in the gastrointestinal tract. Gastric acid tolerance of 73-89% at pH 3.0 and bile salt tolerance of 68-84% at 0.3% oxgall demonstrate superior stress resistance compared to many commercially available probiotic strains [45]. These survival rates exceed the minimum threshold of 50%, typically required for probiotic designation and suggest potential for these strains to reach the intestinal tract in viable numbers following oral consumption [46].

The L. plantarum isolates exhibited particularly impressive acid and bile tolerance, consistent with previous reports characterizing this species as inherently

robust [44,47]. Cell surface hydrophobicity values 36-72% and adhesion capacity of 2.8-8.4 bacteria per Caco-2 cell indicate moderate to strong adhesion potential. These adhesion properties are critical for probiotic functionality as they enable colonization of intestinal mucosa, competitive exclusion of pathogens, and interaction with host immune cells [48].

The antimicrobial activity demonstrated by LAB isolates against multiple pathogenic organisms, including *E. coli, S. aureus*, *S. typhimurium*, and *C. albicans*, represents significant functional value. Importantly, the cell-free supernatants were neutralized to pH 7.0 prior to testing, thereby eliminating the contribution of organic acids to the observed inhibitory effects. This confirms that the antimicrobial activity is attributable to other bioactive metabolites, likely including bacteriocins, hydrogen peroxide, and other proteinaceous compounds produced by the LAB isolates [49-50]. Inhibition zones of 12-24 mm indicate substantial production of these antimicrobial compounds. The superior activity against Gram-positive organisms compared to Gram-negative bacteria is typical of LAB antimicrobials and reflects differences in cell wall structure [18]. Notably, the activity against C. albicans suggests potential application in managing fungal infections and dysbiosis conditions.

The safety assessment revealing the absence of pathogenic bacteria provides reassurance regarding traditional production methods. However, the detection of Enterobacteriaceae at 3.15-4.92 log CFU/g, while within acceptable ranges for fermented foods, highlights the need for good manufacturing practices. The absence of acquired antibiotic resistance in LAB isolates is particularly important for probiotic applications, as transfer of resistance genes to gut microbiota represents a potential public health concern [51].

The spontaneous fermentation approach employed in traditional Dawadawa production relies on indigenous microorganisms from raw materials, fermentation vessels, and the environment. While this method has sustained production for generations, it inherently results in product variability. The identification of specific LAB strains with superior probiotic attributes from this study could facilitate the development of defined starter cultures that maintain traditional characteristics while improving consistency and safety [52].

Bridging to Functional Food Science and Development Pathways: The development of Dawadawa-derived probiotics aligns with contemporary functional food science frameworks emphasizing bioactive compound identification, standardized dosing, and evidence-based health claims [53-54]. Recent advances in functional food research have established systematic pathways for translating traditional fermented foods into validated functional products, requiring comprehensive safety assessment, bioactive quantification, and clinical validation [55]. The probiotic metabolites identified in our LAB isolates—including organic acids, bacteriocins, and exopolysaccharides—represent bioactive compounds with documented immunomodulatory and antimicrobial properties.

Future investigations should quantify specific bioactive concentrations, establish minimum effective doses for health benefits, conduct toxicological assessments following international safety standards, and perform controlled human intervention trials with biomarker endpoints (gut microbiota composition, inflammatory markers, pathogen colonization resistance) to substantiate functional food claims [56-57]. Post-market surveillance addressing stability, shelf-life, and consistent bioactive delivery will be critical for commercial viability [58]. This systematic approach ensures that traditional knowledge is preserved while meeting contemporary food safety and efficacy standards.

Study Limitations and Future Directions: Several limitations warrant acknowledgment. The sample size of thirty may not capture the full extent of regional variation

within Kogi State. Although 16S rRNA gene sequencing provided species-level characterization, whole genome sequencing would enable strain-level differentiation and functional gene profiling. Additionally, in vitro probiotic assessments require validation through vivo studies to confirm physiological effects.

Future research should include comprehensive metagenomic analysis to characterize complete microbial communities, whole-genome sequencing of probiotic strains for detailed genetic characterization, in vivo probiotic efficacy studies using animal models and human clinical trials, and the development and evaluation of starter culture formulations for commercial production.

Scientific Innovations: This investigation provides the first molecular characterization of probiotic LAB from Kogi State Dawadawa, revealing unexpectedly high L. plantarum prevalence (23%) compared to previous reports emphasizing Bacillus dominance. The comprehensive probiotic profiling using standardized in vitro assays (gastric acid/bile tolerance, adhesion, antimicrobial activity) establishes baseline functional parameters for indigenous strains. Notably, the superior acid tolerance (89% at pH 3.0) and adhesion capacity (8.4 CFU/cell) of L. plantarum isolates DWD-05, DWD-12, and DWD-18 exceed many commercial probiotic benchmarks, demonstrating the untapped potential of traditional African fermented foods as sources of novel probiotic strains adapted to West African populations.

Practical Implications: These findings directly benefit traditional Dawadawa producers, food technologists, and public health stakeholders by providing scientific validation of health-promoting claims for this indigenous fermented condiment. The identified L. plantarum strains (DWD-05, DWD-12, DWD-18) can be developed as defined starter cultures to standardize Dawadawa production while maintaining traditional organoleptic characteristics, potentially improving product consistency, safety, and marketability. The demonstrated antimicrobial efficacy against foodborne pathogens supports biopreservation applications in traditional food systems. Furthermore, these indigenous probiotic strains could be formulated into functional food products (fermented beverages, dietary supplements) tailored for local populations, offering culturally appropriate alternatives to imported probiotics. The next translational steps include in vivo efficacy validation using animal models, pilot-scale production trials with traditional producers, and human clinical trials to confirm health benefits.

CONCLUSIONS

This study provides comprehensive characterization of the microbial diversity and probiotic potential of traditionally fermented Dawadawa from Kogi State, Nigeria. The findings demonstrate substantial populations of lactic acid bacteria, particularly Lactobacillus plantarum, with impressive probiotic attributes including gastric acid tolerance, bile salt resistance, and adhesion capacity. The significant antimicrobial efficacy against multiple pathogens confirms the functional properties of these indigenous strains. The microbiological safety assessment supports the continued traditional production and consumption of this indigenous fermented condiment.

These results provide scientific validation of the health-promoting reputation of Dawadawa and lay the foundation for developing standardized production protocols and probiotic products from indigenous sources. The regional variations observed highlight the importance of location-specific characterization studies and preservation of diverse traditional fermentation practices. The identified LAB strains, particularly L. plantarum isolates DWD-05, DWD-12, and DWD-18, demonstrate exceptional probiotic potential and warrant further investigation for commercial probiotic development.

Conflicts Of Interest: The authors declare no conflicts of interest in relation to this research.

Authors' Contributions: **Z.D.A.**: Conceptualization, methodology, investigation, formal analysis, data curation, writing—original draft preparation, project administration.

J.A-O.: Investigation, methodology, laboratory analysis, microbiological enumeration, data collection.

A.A.M.: Conceptualization, validation, resources, writing—review and editing, supervision, project administration, corresponding author responsibilities.

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REFERENCES

1. Tamang JP, Watanabe K, Holzapfel WH. Review: Diversity of microorganisms in global fermented foods and beverages. *Front Microbiol*. 2016;7:377. DOI: https://doi.org/10.3389/fmicb.2016.00377
2. Marco ML, Sanders ME, Gänzle M, Arrieta MC, Cotter PD, De Vuyst L, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on fermented foods. *Nat Rev Gastroenterol Hepatol*. 2021;18(3):196-208. DOI: https://doi.org/10.1038/s41575-020-00390-5
3. Şanlier N, Gökcen BB, Sezgin AC. Health benefits of fermented foods. *Crit Rev Food Sci Nutr*. 2019;59(3):506-527. DOI: https://doi.org/10.1080/10408398.2017.1383355
4. Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SR. Chemical and biochemical changes in African locust bean (Parkia biglobosa) and melon (Citrullus vulgaris) seeds during fermentation to condiments. *Pak J Nutr*. 2004;3(3):140-145. DOI: https://doi.org/10.3923/pjn.2004.140.145
5. Oguntoyinbo FA, Cho GS, Trierweiler B, Kabisch J, Rösch N, Neve H, et al. Fermentation of African locust bean (Parkia biglobosa) seeds: microbial diversity and impact on nutritional parameters. *Food Microbiol*. 2016;56:1-10. DOI: https://doi.org/10.1016/j.fm.2015.11.015
6. Enujiugha VN, Akanbi CT, Adeniran HA. Evaluation of the protein quality of African oil bean seed (Pentaclethra macrophylla Benth) protein isolate. *Pak J Nutr*. 2005;4(6):374-378.

DOI: https://doi.org/10.3923/pjn.2005.374.378

7. Parkouda C, Nielsen DS, Azokpota P, Iversen JJL, Hounhouigan JD, Jensen JS, et al. The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. *Crit Rev Microbiol*. 2009;35(2):139-156. DOI: https://doi.org/10.1080/10408410902793056
8. Sanni AI, Onilude AA, Fadahunsi IF, Afolabi RO. Microbial deterioration of traditional fermented sorghum gruel (ogi) and its control. *World J Microbiol Biotechnol*. 1999;15(3):295-298. DOI: https://doi.org/10.1023/A:1008987930229
9. Ouoba LII, Rechinger KB, Barkholt V, Diawara B, Traore AS, Jakobsen M. Degradation of proteins during the fermentation of African locust bean (Parkia biglobosa) by strains of Bacillus subtilis and Bacillus pumilus for production of Soumbala. *J Appl Microbiol*. 2003;94(3):396-402. DOI: https://doi.org/10.1046/j.1365-2672.2003.01845.x
10. Azokpota P, Hounhouigan DJ, Nago MC. Microbiological and chemical changes during the fermentation of African locust bean (Parkia biglobosa) to produce afitin, iru and sonru, three traditional condiments produced in Benin. *Int J Food Microbiol*. 2006;107(3):304-309. DOI: https://doi.org/10.1016/j.ijfoodmicro.2005.10.026
11. Beaumont M. Flavouring composition prepared by fermentation with Bacillus spp. *Int J Food Microbiol*. 2002;75(3):189-196. DOI: https://doi.org/10.1016/S0168-1605(01)00706-1
12. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514. DOI: https://doi.org/10.1038/nrgastro.2014.66
13. Sánchez B, Delgado S, Blanco-Míguez A, Lourenço A, Gueimonde M, Margolles A. Probiotics, gut microbiota, and their influence on host health and disease. *Mol Nutr Food Res*. 2017;61(1):1600240. DOI: https://doi.org/10.1002/mnfr.201600240
14. Ogunshe AAO, Omotoso MA, Kolawole OM. Microbial studies and biochemical characteristics of controlled fermented Aframomum danielli, K. Schum, a tropical spice. *Malays J Microbiol*. 2006;2(1):29-36
15. Oguntoyinbo FA, Tourlomousis P, Gasson MJ, Narbad A. Analysis of bacterial communities of traditional fermented West African cereal foods using culture independent methods. *Int J Food Microbiol*. 2011;145(1):205-210. DOI: https://doi.org/10.1016/j.ijfoodmicro.2010.12.025
16. Omafuvbe BO, Shonukan OO, Abiose SH. Microbiological and biochemical changes in the traditional fermentation of soybean for 'soy-daddawa' - Nigerian food condiment. *Food Microbiol*. 2000;17(5):469-474. DOI: https://doi.org/10.1006/fmic.1999.0332
17. Morelli L, Capurso L. FAO/WHO guidelines on probiotics: 10 years later. *J Clin Gastroenterol*. 2012;46 Suppl:S1-2. DOI: https://doi.org/10.1097/MCG.0b013e318269fdd5
18. Zakari D, Amoka A, Idris E, Anoze A, Moyosore A, Boniface M, et al. Characterization of lactic acid bacteria from fermented cereal-based foods in Anyigba, Nigeria, for potential probiotic and bio-preservation applications. *Agriculture and Food Bioactive Compounds*. 2025;2(8):171-182. DOI: https://doi.org/10.31989/afbc.v2i8.1894
19. Franz CMAP, Huch M, Abriouel H, Holzapfel W, Gálvez A. Enterococci as probiotics and their implications in food safety. *Int J Food Microbiol*. 2011;151(2):125-140. DOI: https://doi.org/10.1016/j.ijfoodmicro.2011.08.014
20. Harrigan WF, McCance ME. Laboratory methods in food and dairy microbiology. London: Academic Press; 1976
21. International Organization for Standardization. ISO 4833-1:2013. Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique. Geneva: ISO; 2013
22. De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. *J Appl Bacteriol*. 1960;23(1):130-135. DOI: https://doi.org/10.1111/j.1365-2672.1960.tb00188.x
23. Claus D, Berkeley RCW. Genus Bacillus Cohn 1872. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG, editors. Bergey's manual of systematic bacteriology. Volume 2. Baltimore: Williams and Wilkins; 1986:1105-1139
24. International Organization for Standardization. ISO 21528-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count technique. Geneva: ISO; 2017
25. Beuchat LR, Cousin MA. Yeasts and molds. In: Downes FP, Ito K, editors. Compendium of methods for the microbiological examination of foods. 4th ed. Washington DC: American Public Health Association; 2001:209-215
26. Cowan ST, Steel KJ. Manual for the identification of medical bacteria. 3rd ed. Cambridge: Cambridge University Press; 1993
27. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams and Wilkins; 1994
28. Axelsson L. Lactic acid bacteria: classification and physiology. In: Salminen S, Von Wright A, Ouwehand A, editors. Lactic acid bacteria: microbiological and functional aspects. 3rd ed. New York: Marcel Dekker; 2004:1-66

29. Dashti AA, Jadaon MM, Abdulsamad AM, Dashti HM. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. *Kuwait Med J*. 2009;41(2):117-122
30. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol*. 1991;173(2):697-703. DOI: https://doi.org/10.1128/jb.173.2.697-703.1991
31. Garbeva P, Van Overbeek LS, Van Vuurde JWL, Van Elsas JD. Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microb Ecol*. 2001;41(4):369-383. DOI: https://doi.org/10.1007/s002480000096
32. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990;215(3):403-410. DOI: https://doi.org/10.1016/S0022-2836(05)80360-2
33. Charteris WP, Kelly PM, Morelli L, Collins JK. Development and application of an in vitro methodology to determine the transit tolerance of potentially probiotic Lactobacillus and Bifidobacterium species in the upper human gastrointestinal tract. *J Appl Microbiol*. 1998;84(5):759-768. DOI: https://doi.org/10.1046/j.1365-2672.1998.00407.x
34. Havenaar R, Huis In't Veld JHJ. Probiotics: a general view. In: Wood BJB, editor. The lactic acid bacteria. Volume 1: The lactic acid bacteria in health and disease. London: Elsevier Applied Science; 1992:151-170
35. Rosenberg M, Gutnick D, Rosenberg E. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol Lett*. 1980;9(1):29-33. DOI: https://doi.org/10.1111/j.1574-6968.1980.tb05599.x
36. Del Re B, Sgorbati B, Miglioli M, Palenzona D. Adhesion, autoaggregation and hydrophobicity of 13 strains of Bifidobacterium longum. *Lett Appl Microbiol*. 2000;31(6):438-442. DOI: https://doi.org/10.1046/j.1365-2672.2000.00845.x
37. Zakari AD, Audu GA, Egbeja TI, Aliyu AA, Adefila MA, Momoh TB, et al. Antioxidant and hepatoprotective activities of methanol extract of Moringa oleifera leaves in carbon tetrachloride-induced hepatotoxicity in rats: Implications for functional food development. *Agriculture and Food Bioactive Compounds*. 2025;2(7):157-168. DOI: https://doi.org/10.31989/afbc.v2i7.1722
38. Schillinger U, Lücke FK. Antibacterial activity of Lactobacillus sake isolated from meat. *Appl Environ Microbiol*. 1989;55(8):1901-1906. DOI: https://doi.org/10.1128/aem.55.8.1901-1906.1989
39. International Organization for Standardization. ISO 6579-1:2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp. Geneva: ISO; 2017
40. United States Food and Drug Administration. Bacteriological analytical manual. Chapter 10: Detection of Listeria monocytogenes in foods and environmental samples, and enumeration of Listeria monocytogenes in foods. Silver Spring: FDA; 2021
41. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 30th ed. CLSI supplement M100. Wayne: CLSI; 2020
42. Kostinek M, Specht I, Edward VA, Schillinger U, Hertel C, Holzapfel WH, et al. Diversity and technological properties of predominant lactic acid bacteria from fermented cassava used for the preparation of Gari, a traditional African food. *Syst Appl Microbiol*. 2005;28(6):527-540. DOI: https://doi.org/10.1016/j.syapm.2005.03.001
43. Nwachukwu E, Achi OK, Ijeoma IO. Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods. *Afr J Food Agric Nutr Dev*. 2010;10(4):2372-2388. DOI: https://doi.org/10.4314/ajfand.v10i4.56338
44. De Vries MC, Vaughan EE, Kleerebezem M, De Vos WM. Lactobacillus plantarum - survival, functional and potential probiotic properties in the human intestinal tract. *Int Dairy J*. 2006;16(9):1018-1028. DOI: https://doi.org/10.1016/j.idairyj.2005.09.003
45. Jacobsen CN, Nielsen VR, Hayford AE, Møller PL, Michaelsen KF, Paerregaard A, et al. Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. *Appl Environ Microbiol*. 1999;65(11):4949-4956. DOI: https://doi.org/10.1128/AEM.65.11.4949-4956.1999
46. Dunne C, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, et al. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am J Clin Nutr*. 2001;73(2 Suppl):386S-392S. DOI: https://doi.org/10.1093/ajcn/73.2.386s
47. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev*. 2008;72(4):728-764. DOI: https://doi.org/10.1128/MMBR.00017-08
48. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Host interactions of probiotic bacterial surface molecules: comparison with commensals and pathogens. *Nat Rev Microbiol*. 2010;8(3):171-184. DOI: https://doi.org/10.1038/nrmicro2297
49. Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol*. 2001;71(1):1-20. DOI: https://doi.org/10.1016/S0168-1605(01)00560-8

50. Ouwehand AC. Antimicrobial components from lactic acid bacteria. In: Salminen S, von Wright A, Ouwehand A, editors. Lactic acid bacteria: microbiological and functional aspects. 3rd ed. New York: Marcel Dekker; 2004:375-395
51. Gueimonde M, Sánchez B, de Los Reyes-Gavilán CG, Margolles A. Antibiotic resistance in probiotic bacteria. *Front Microbiol*. 2013;4:202. DOI: https://doi.org/10.3389/fmicb.2013.00202
52. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol*. 2004;15(2):67-78. DOI: https://doi.org/10.1016/j.tifs.2003.09.004
53. Miyasaka K, Yoshida Y, Martirosyan D. Functional food development: from bioactive discovery to clinical validation. *Functional Foods in Health and Disease*. 2025;15(8):506-518. DOI: https://doi.org/10.31989/ffhd.v15i8.1666
54. Martirosyan D. Bioactive compounds in fermented foods: mechanistic insights and therapeutic potential. *Bioactive Compounds in Health and Disease*. 2025;8(6):218-229. DOI: https://doi.org/10.31989/bchd.v8i6.1667
55. Chen W, Liu Y, Martirosyan D. Safety assessment frameworks for traditional fermented foods: from laboratory to market. *Bioactive Compounds in Health and Disease*. 2024;7(11):512-530. DOI: https://doi.org/10.31989/bchd.v7i11.1534
56. Rithi AT, Johnson M, Chen L. Systematic approaches to functional food formulation: dose optimization and biomarker validation. *Functional Food Science*. 2023;4(1):11-28. DOI: https://doi.org/10.31989/ffs.v4i1.1271
57. Rodriguez-Martinez C, Kim JH, Anderson PL. Clinical trial design for functional foods: biomarker selection and endpoint validation. *Functional Food Science*. 2024;3(6):245-262. DOI: https://doi.org/10.31989/ffs.v3i6.1398
58. Martinez-Lopez E, Ogunremi OR, Silva KR. Post-market surveillance and quality assurance in probiotic functional foods. *Dietary Supplements and Nutraceuticals*. 2024;3(9):78-93. DOI: https://doi.org/10.31989/dsn.v3i9.1467
59. Zakari DA, Olateju KS, Eneye BK, Amoka AG, Anoze AA. Hurdle concept and the role of lactic acid bacteria in food preservation. *Journal of Food Technology and Nutrition Sciences*. 2020;6(4):2-7
60. Xie B, Wang R, Martinez A. Standardization of probiotic supplements: quality control and post-market surveillance. *Dietary Supplements and Nutraceuticals*. 2025;4(6):1-11. DOI: https://doi.org/10.31989/dsn.v4i6.1621