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In Vitro Probiotic Characterization of *Lactiplantibacillus plantarum* Strains Isolated from Traditional Fermented Dockounou Paste

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Abstract: This study aimed to evaluate the probiotic properties of 10 lactic acid bacteria (LAB) isolated from artisanal fermented plantain dockounou paste. A preliminary characterization of the LAB isolates was performed based on phenotypic and several biochemical properties, which was subsequently confirmed through 16S rRNA gene sequencing analysis, indicating that these isolates belonged to the species *Lactiplantibacillus plantarum*. With regard to safety criteria, the strains exhibited no alpha or beta hemolysis activity. Nevertheless, the majority of LAB strains demonstrated high sensitivity to the antibiotics tested. The results demonstrated that the majority of the strains exhibited remarkably high survival rates under simulated gastrointestinal conditions, such as pH = 1.5 (81.18–98.15%), 0.3% bile salts (68.62–100.89%), 0.4% phenol (40.59–128.24%), as well as 0.1% pepsin and pH = 2.5 (88.54–99.78%). The LAB strains demonstrated elevated levels of cell surface properties, indicative of the presence of a considerable defensive mechanism against pathogens. Intact LAB cells exhibited significant antioxidant abilities (48.18–83.58%). They also demonstrated a pronounced inhibitory effect on the growth of foodborne pathogens. Enzyme pattern analysis revealed that the LAB isolates produced both proteases and cellulases, as well as pectinase and/or amylase activity. The potential of the *L. plantarum* strains FS43, FS44, and FS48, as indicated by the results obtained from the standard *in vitro* assays, makes them suitable for further study as potential probiotics.

Keywords: fermented plantain dockounou paste; *Lactiplantibacillus plantarum*; probiotic properties; health benefits

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1. Introduction

Dockounou paste is an Ivorian traditional food made by combining senescent plantain flesh with maize or rice flour and baking or steaming it [1,2]. This food is produced through several processes, including peeling the senescent plantain fruit, scooping out the flesh, combining it with flour, fermenting, packing, frying, and processing it into flour [1,2].

Lactic acid bacteria, including those belonging to the genera *Lactococcus*, *Lactobacillus*, and *Streptococcus*, have been identified as playing a key role in the fermentation of traditional cereal-based foods and beverages worldwide [3–6]. Fermentation is the most significant of these processes since it improves the texture and organoleptic qualities of the food [4–6].

The potential health benefits of indigenous African fermented foods and beverages have not been adequately researched. It is a widely held view that the popularity of tradi-

tional fermented foods can be attributed to many factors, including the enhancement of taste, the conservation of food for a longer shelf life, and the improvement of nutritional value and digestive effectiveness. Some African cultural groups espouse the belief that fermented foods can be efficient in the treatment of illnesses, with a particular focus on digestive disorders. The indigenous microbial consortia present in fermented indigenous African foods are responsible for the beneficial properties of fermented foods mentioned above (reviewed by Achi and Asamudo [7]; Malongane and Berejena [8]). In recent years, numerous studies have been conducted with a focus on the isolation of LABs and yeasts from traditional fermented foods to identify potential starter cultures that can also exhibit valuable probiotic properties [3–6]. LAB species have been identified as the agents responsible for the manufacture of various Ivorian indigenous foods and beverages, such as *attiéké*, *attoupkou*, *placali* (fermented tubers), *baca*, *wômi*, *doklu* (fermented cereal-based foods), *adjuevan* (fermented fish), *bandji* and *tchapalo* (fermented beverages), and *dockounou* (fermented over-ripe fruits of plantain) [8–14]. Throughout the fermentation process of dockounou paste, Kouadio et al. [11] found different types of microorganisms predominating, such as yeasts, bacilli, and lactic acid bacteria. *L. plantarum* (64%), *Weissella cibaria* (22%), *L. fermentum* (7%), and *Pediococcus acidilactici* (7%) were mainly detected during the first 24 h of the production of doklu. *L. plantarum* (56%) dominated after 48 h of fermentation, and *L. fermentum* (100%) prevailed at the end of fermentation [12]. Mogmenga et al. [15] successfully isolated *Saccharomyces cerevisiae* with proven probiotic properties from a traditional fermented beer produced in Burkina Faso, known as Rabilé.

Also, the presence of *Lactobacillus* spp., *Bifidobacterium* spp., and *S. cerevisiae* yeasts has been identified in Romanian fermented beverages (socata and borș) [16,17], Romanian fermented dairy products [18–20], and Romanian fermented vegetables [18,21–23].

Probiotics are live microorganisms that assist the body in maintaining optimal health when consumed in appropriate quantities [24,25]. Lactic acid bacteria, such as *Bifidobacterium* and *Lactobacillus*, isolated from various sources, have been widely investigated using standard *in vitro* tests for key functional, probiotic, and technological properties [26–32]. The beneficial effects on health can be attributed to the synthesis of compounds that inhibit the proliferation of pathogens and compete with them for nutrients and adherence sites on the epithelial cells of the gut tract [29–32]. Probiotics can improve the immune system through macrophage activation, increased levels of immune globulins, increased natural killer cell activity, and/or increased levels of cytokines [30–32].

Furthermore, pharmaceutical probiotics are often expensive, and fermented foods and beverages represent a valuable alternative source of probiotics that may be more accessible to those who are unable to afford them, particularly in marginalized communities in Africa.

The objective of the present study was to assess the potential of LAB strains isolated from traditional fermented plantain dockounou paste as a probiotic *in vitro*. This was achieved by evaluating their functional properties (resistance in simulated gastrointestinal conditions and cell surface properties), probiotic properties (antibacterial, antioxidant, and enzyme activities), and safety properties (molecular identification, antibiotic sensitivity, and hemolysis activity).

2. Materials and Methods

2.1. LAB Strains and Growth Conditions

To evaluate their probiotic properties, 40 bacterial strains were isolated from fermented Dockounou paste [11]. The LAB isolates were preliminarily identified using conventional techniques, including colonial morphology, Gram staining, and biochemical reactions (catalase and oxidase tests). A total of 10 representative LAB isolates were selected for this research and preserved in cryotubes at a temperature of −20 °C in MRS broth, which was additionally supplemented with 20% glycerol. Before use, the stock LAB cultures were inoculated into MRS broth and incubated at 37 °C for 24 h.

2.2. Identification of LAB Strains via 16S rDNA Sequencing

Overnight LAB cultures were harvested through centrifugation at 5000× *g* for 10 min. The LAB cells were then employed to isolate bacterial DNA using the Zymo Research Quick-DNA™ Fungal/Bacterial Miniprep Extraction Kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. Finally, DNA quantification was conducted using the SpectraMax®® QuickDrop™ (Molecular Devices, San Jose, CA, USA). Polymerase chain reaction (PCR) amplification of the 16S ribosomal DNA (rDNA) region was performed using the primers 27F (5′ -AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′ -TACGGYTACCTTGTTACGACTT-3′). Amplified DNA fragments were then subjected to sequencing in both directions using the same primers by the Cellular and Molecular Immunological Application (CEMIA) sequencing service (Larissa, Greece). The newly obtained sequences were subjected to comparison with those already deposited in the NCBI database using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi, latest accessed 4 December 2023) to identify the species level of the LAB strains based on the level of similarity. Furthermore, a phylogenetic tree was constructed using MEGA software version 11.0.13 [33] based on 16S rDNA genes to identify the bacterial species with the highest degree of similarity [34].

2.3. In Vitro *Tolerance of LAB Strains to Simulated Gastrointestinal Conditions*

2.3.1. Resistance to Low pH

The influence of low pH (1.5) on the growth of LAB strains was explored using the method described by Carr et al. [35]. This involved inoculating fresh bacterial cultures into 6 mL of sterile MRS broth adjusted to pH 1.5 with 1 M HCl and incubating them at 37 °C for 3 h. The number of viable cells was then determined using the plate count agar method, with the results expressed as log CFU/mL. Survival rate (SR) was calculated using the following formula:

$$\mathrm{SR}\% = \frac{\mathrm{Log\ CFU/mL(finale)}}{\mathrm{Log\ CFU/mL(initial)}} \times 100, \quad (1)$$

where initial and final mean the viable cells (CFU/mL) at 0 h and after 3 h of incubation.

2.3.2. Resistance to Pepsin and Low pH

The capacity of LAB isolates to tolerate pepsin in acid media was tested following the method proposed by Diguță et al. [36], with few modifications. The simulated gastric juice was formulated by combining 0.128% sodium chloride (NaCl), 0.0239% potassium chloride (KCl), and 0.64% sodium bicarbonate ($NaHCO_3$) (Central Drug House, Mumbai, India) with 0.1% (*w*/*v*) pepsin (Himedia, Mumbai, India). The pH was then adjusted to 2.5. Overnight LAB cells were collected via centrifugation at 2000× *g* for 10 min and washed twice in sterile physiological saline (0.9% NaCl). The cells were then suspended in simulated gastric juice. Samples were taken at 0 and 3 h, serially diluted, and plated on MRS agar plates. Viable cells were quantified using the plate count method. The formula employed to calculate the survival rate in simulated gastric juice is identical to that used to assess acid pH resistance.

2.3.3. Tolerance to Bile Salts

The methodology proposed by Coulibaly et al. [28] was employed to assess the ability of LAB strains to withstand the effects of bile salts. The LAB strains were prepared as previously described. Cell pellets were inoculated in MRS broth supplemented with 0.3% (*w*/*v*) bile salts and then incubated for 4 h at 37 °C. Viable cells were quantified using

the plate count method, with results expressed as log CFU/mL. The survival rate was performed according to the following formula:

$$SR\% = \frac{\text{Log CFU/mL(final)}}{\text{Log CFU/mL(initial)}} \times 100, \quad (2)$$

where initial and final mean the viable cells (CFU/mL) at 0 h and after 4 h of incubation.

2.3.4. Phenol Tolerance

The ability of LAB isolates to survive in the presence of a phenol solution was investigated using a methodology described by Xanthopoulos et al. [37]. Overnight LAB cultures were inoculated in MRS broth containing 0.4% phenol and incubated for 24 h at 37 °C. Viable cells were subsequently quantified via the plate count method.

2.4. Safety Assessment

2.4.1. Hemolysis Test

The hemolytic potential of LAB isolates was performed by the methodology described by Yadav et al. [38]. Bacterial strains were inoculated onto blood agar plates (Oxoid, Basingstoke Hampshire, UK) supplemented with 5% (*w*/*v*) sheep blood and incubated at a temperature of 37 °C for 48 h. If the area around the bacterial growth was clear, it can be assumed that the bacterium had caused β-hemolysis. Conversely, if the area had turned greenish, this would indicate that the tested bacterium had produced α-hemolysis. It should be noted that γ-hemolysis is the term used to describe the absence of hemolysis or blood cell degradation when a microorganism is present.

2.4.2. Antibiotic Sensitivity

The antibiotic spectrum of the LAB isolates was evaluated using the disc diffusion method with antibiotics aligned with those recommended by the European Food Safety Authority (EFSA). This included chloramphenicol (30 µg/disc); tetracycline (30 µg/disc); erythromycin (10 µg/disc); lincomycin (10 µg/disc); ampicillin ((10 µg/disc); amoxicillin + clavulanic acid (20/10 µg/disc); penicillin (2 µg/disc); nitrofurantoin (300 µg/disc); trimethoprim + sulfamethoxazole (1/19 µg/disc). A 100 µL aliquot of each fresh LAB culture was spread onto MRS agar plates and allowed to dry. Subsequently, antibiotic discs were placed on the inoculated plates and incubated for 48 h at 37 °C. The diameter of the clear zone surrounding each disc was measured in millimeters to determine the antibiotic susceptibility of the isolates. Subsequently, the results were interpreted following the established guidelines set by the Clinical and Laboratory Standards Institute (CLSI) [39]. Isolates exhibiting diameters ≥20 mm were classified as susceptible (S), while those with diameters in the range of 15–20 mm were classified as intermediate (I), and those with diameters <15mm were classified as resistant (R).

2.5. Cell Surface Characteristics

2.5.1. Co-Aggregation Test

The co-aggregation capacities were evaluated using the methodology proposed by Collado et al. [40]. Overnight LAB cultures were harvested via centrifugation at 4000 rpm for 10 min and adjusted to a concentration of 10^8 CFU/mL using PBS buffer (VWR International, Rosny-sous-Bois, France). The target pathogens employed were *Escherichia coli* ATCC 8739 and *Salmonella enterica* serovar Typhimurium ATCC 14028. The indicator pathogens were cultivated in Luria–Bertani broth (Tulip Diagnostics (P) Ltd., Verna, Goa, India) overnight and prepared as described above. The LAB cell suspension (2 mL) was combined with a corresponding volume of pathogenic bacteria suspension. This mixture

was vortexed for 10 s to ensure thorough homogenization. The mixture was then incubated at 37 °C for 4 h. The co-aggregation capacity was expressed as follows:

$$\% \text{ co-aggregation} = \frac{\text{Amix0} - \text{Amix}}{\text{Amix0}} \times 100, \quad (3)$$

where Amix0 represents the absorbance of the bacterial mixture at t = 0, while Amix represents the absorbance of the same mixture after 4 h of incubation.

2.5.2. Cell Surface Hydrophobicity

The cell surface hydrophobicity was determined according to the method developed by Rosenberg et al. [41], with a few modifications. For this purpose, bacterial cells in the stationary phase were centrifuged at 10,000× *g* for 5 min, washed twice with phosphate-buffered saline (PBS: 130 mM sodium chloride, 10 mM sodium phosphate, pH 7.2), and then resuspended in PBS buffer. The absorbance (A0) was measured at 600 nm and standardized to 0.25 ± 0.05. A 3 mL portion of the cell suspension and 1 mL of hydrocarbon (xylene) were mixed and vigorously vortexed for 2 min. Following this, the suspension was incubated at 37 °C for one hour without the application of any shaking to facilitate the separation of the aqueous and organic phases. Subsequently, the aqueous phase was carefully gathered, and the absorbance was quantified at 600 nm (A1). The percentage of bacterial adhesion to the solvent was calculated using the following formula:

$$\% \text{ cell surface hydrophobicity} = \frac{\text{A1}}{\text{A0}} \times 100. \quad (4)$$

2.5.3. Auto Aggregation Test

The capacity of LAB isolates to aggregate spontaneously was evaluated using the methodology delineated by Rosenberg et al. [41]. The overnight culture was collected via centrifugation at 4000 rpm for 10 min at 4 °C, washed twice with PBS and resuspended in PBS buffer. The LAB suspensions were incubated at 37 °C for 24 h. The optical density of the bacterial suspension was measured at 600 nm. The percentage of auto-aggregation was calculated using the following equation:

$$\% \text{ auto-aggregation} = 1 - \frac{\text{At}}{\text{A0}} \times 100, \quad (5)$$

where At is the absorbance after 24 h, and A0 is the absorbance t = 0.

2.6. Study of the Probiotic Properties of Lactic Acid Bacteria

2.6.1. Antibacterial Activity

The antibacterial activity of LAB isolates was tested against four reference pathogens, including Gram-positive bacteria (*Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 33592) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Salmonella enterica* serovar Typhimurium ATCC 14028). This was conducted using the agar well diffusion method, as described by Balouiri et al. [42], with minor modifications. LAB isolates were cultivated in MRS broth at 37 °C for 48 h. Subsequently, the cell-free supernatants (CFSs) were obtained from the LAB isolates via centrifugation at 10,000 rpm for 10 min at 4 °C and filtration through sterile 0.22-micron Millipore filters (VWR International, Rosny-sous-Bois, France). An overnight pathogenic culture was prepared by adjusting the optical density at 600 nm (OD600 nm) to 0.2 ± 0.05 units (corresponding to a concentration of approximately 10^7 to 10^8 CFU/mL). The next stage was to transfer 1 mL of the pathogenic suspension to a sterile Petri plate (90 mm) and covered with 20 mL of TSA

(Scharlab S.L., Barcelona, Spain), which was cooled to 45 °C. The suspension was then homogenized gently until solidified. A sterile tip was employed in an aseptic manner to puncture 6 mm-diameter wells. Thereafter, a volume of 100 µL of the CFSs was added to each well. The plate was incubated at 37 °C for 24 h. The presence of a clear zone of 1 mm or more surrounding each well confirmed positive inhibition, which indicates antibacterial activity.

2.6.2. DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging activity was assessed following the method outlined by Brand-Williams et al. [43]. Specifically, 0.2 mL of a freshly prepared solution of DPPH (0.2 mM) in ethanol was added to 0.8 mL of the sample solution (supernatant or intact cells). The reaction mixture was thoroughly vortexed and then incubated at room temperature in the dark for 30 min. The measurement of absorbance took place at 517 nm against an ethanol-containing blank. The DPPH solution-free sample was used as a positive control. The scavenging capacity is then calculated as follows:

$$\% \text{ Scavenger effect} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100. \quad (6)$$

2.6.3. Plate Screening of LAB Isolates for Hydrolytic Enzymes

The enzymatic patterns were assessed by inoculating LAB strains as spots on Luria agar (Tulip Diagnostics (P) Ltd., Verna, Goa, India) supplemented with different carbon sources such as 2% of soluble starch (amylase), 2% carboxymethylcellulose (cellulase), 2% olive oil (lipase), 2% and pectin (pectinase), as described by Coulibaly et al. [28] and Proca et al. [44] with slight modifications. To detect protease activity, skim milk (0.1% fat) was added in a 1:2 ratio with water (*v*/*v*) and 2% agar. The prepared plates were then incubated at 37 °C for 48 h. Amylase activity was revealed using Lugol solution (Tody Laboratories Int. S.R.L., Bucharest, România). A clear zone surrounding the bacterial isolate indicated a positive reaction. Cellulase activity was visualized as a clear halo surrounding bacterial growth by staining with a 0.1% Congo red solution (VWR Bdh Chemicals, Leuven, Belgium) and then washed with 1 M NaCl. A precipitation zone around the colonies was identified as lipase producers. Bacterial strains that developed a clear halo after flooding with Lugol solution indicated the presence of pectinase activity. Bacterial isolates that showed a clear zone of casein degradation were considered positive for protease production.

2.7. Statistical Analysis

The experiments were conducted in triplicate, and the results were expressed as the mean ± standard deviation. Excel 2016 was used for calculations, figures, and box plots. To select the best isolates, principal component analysis (PCA) and ascending hierarchical classification (AHC) were performed using XLStat (Version 2016) software.

3. Results

3.1. Identification of LAB Isolates

Ten distinct colonies were isolated from four dockounou-fermented pastes using classical methods. After growing on the MRS agar surface, the LAB isolates displayed smooth, round, cream-white colonies. Phenotypic characterization revealed rod-shaped cells that were Gram-positive and catalase-negative, as well as oxidase-negative (Table 1).

Sequence homology was demonstrated to range from 98% to 99% with 16S rDNA sequences from reference strains within the NCBI database. This led to the identification of the LAB isolates as belonging to the *Lactiplantibacillus plantarum*. A phylogenetic tree was created using MEGA (Molecular Evolution Genetic Analysis) software, version Xto display identities and relationships of representative and related standard strains (Figure 1). The partial 16S rDNA sequences from the LAB strains have been submitted to the NCBI database under accession codes PP196396 (*Lactiplantibacillus plantarum* FS43P4), PP196397 (*L. plantarum* FS44P4), PP196398 (*L. plantarum* FS45P4), PP196399 (*L. plantarum* FS46P4),

PP196400 (*L. plantarum* FS47P4), PP196401 (*L. plantarum* FS48P4), PP196402 (*L. plantarum* FS49P4), PP196403 (*L. plantarum* FS50P4), PP196404 (*L. plantarum* FS51P4), and PP196405 (*L. plantarum* FS65P4).

Table 1. Phenotypic characteristics of the LAB strains used in this study.

Isolates	Parameters					Origin of Isolate
	Shape	Gram Reaction	Cell	Catalase Test	Oxidase Test	
FS43	Smooth	+	rod-shape	-	-	Rice dockounou paste
FS51	Round	+	rod-shape	-	-	Rice dockounou paste
FS50	Smooth	+	rod-shape	-	-	Maize dockounou paste
FS49	Round	+	rod-shape	-	-	Maize dockounou paste
FS65	Smooth	+	rod-shape	-	-	Maize dockounou paste
FS48	Smooth	+	rod-shape	-	-	Maize dockounou paste
FS47	Round	+	rod-shape	-	-	Millet dockounou paste
FS46	Round	+	rod-shape	-	-	Millet dockounou paste
FS45	Smooth	+	rod-shape	-	-	Millet dockounou paste
FS44	Round	+	rod-shape	-	-	Cassava dockounou paste

Figure 1. Molecular phylogenetic analysis via maximum parsimony analysis of taxa.

3.2. Safety Criteria of LAB Strains

When assessing the safety of potential probiotic strains *in vitro*, antibiotic resistance and hemolytic activity are key criteria (refer to Table 2). All LAB strains that were tested were susceptible to the amoxicillin–clavulanic acid complex, nitrofurantoin, chloramphenicol, ampicillin, and sulfonamide–diaminopyrimidine complex. Only strain FS43 showed high susceptibility to the tetracycline class, while strains FS51, FS46, and FS44 showed intermediate susceptibility. Among the macrolide class, only strains FS50, FS47, and FS44 demonstrated resistance to lincomycin. Concerning erythromycin, strains FS50, FS47, and FS46 were susceptible when the diameter was over 20 mm, whereas strains FS65, FS48, FS45, FS44, FS51, and FS49 showed intermediate susceptibility. In the presence of penicillin, only strain FS65 was resistant, while the other strains showed either intermediate or complete sensitivity. The complex of sulphonamide and diaminopyrimidine exhibited intermediate sensitivity only in FS43, whereas the other strains were sensitive to this antibiotic. Additionally, none of the selected strains demonstrated hemolytic activity (refer to Table 2).

Table 2. Antibiotic sensibility and Hemolysis activity of LAB strains.

Strains / Class/Antibiotics		FS43	FS51	FS50	FS49	FS65	FS48	FS47	FS46	FS45	FS44
Chloramphenicol	CHL	20S	24S	24S	28S	24S	24S	26S	25S	24S	29S
Tetracyclin	T	20S	16I	14R	12R	12R	14R	12R	16I	14R	18I
Macrolides	E	12R	18I	20S	19I	18I	18I	21S	20S	18I	16I
	L	26S	24S	0R	26S	20S	26S	0R	28S	30S	0R
Beta-lactam	AM	20S	24S	26S	22S	20S	20S	24S	22S	22S	20S
	AMC	18S	20S	26S	30S	30S	28S	34S	32S	28S	26S
	P	16I	18I	18I	16I	14R	20S	20S	18I	20S	16I
Nitrofuran	F	22S	28S	22S	26S	22S	25S	28S	29S	28S	24S
Sulfamide + diaminopyrimidine	SXT	18I	20S	20S	22S	20S	22S	20S	20S	20S	20S
Hemolysis activity		γ	γ	γ	γ	γ	γ	γ	γ	γ	γ

CHL: Chloramphenicol (30 µg/disc); T: Tetracycline (30 µg/disc); E: Erythromycin (10 µg/disc); L: Lincomycin (10 µg/disc); AM: Ampicillin ((10 µg/disc); AMC: Amoxicillin + clavulanic acid (20/10 µg/disc); P: Penicillin (2 µg/disc); F: Nitrofurantoin (300 µg/disc); SXT: Trimethoprim + sulfamethoxazole (1/19 µg/disc). S: sensitive; I: intermediate; R: resistant; γ—gamma hemolysis.

3.3. Exploration of Probiotic Properties

3.3.1. Resistance to Simulated Gastrointestinal Tract Conditions

This study found that the survival of LAB strains varied depending on the stress conditions. Tolerance to high acidity levels (pH 1.5) in the stomach is a crucial prerequisite for LAB isolates to function as probiotics. As shown in Table 3, strain FS49 had the lowest survival rate (81.18 ± 1.05%), while strain FS65 had the highest (98.15 ± 0.95%). Furthermore, all LAB strains in this study demonstrated excellent growth at pH 2.5 and 0.1% pepsin, as shown in Table 3. The results indicate that LAB isolates exhibit remarkable resilience to harsh gastric conditions. Upon exposure to 0.4% phenol, the majority of strains (FS43, FS44, FS45, FS46, FS47, FS48, and FS49) demonstrated a survival rate exceeding 70%. Following exposure to 0.3% bile salts for 4 h, all LAB strains tested exhibited excellent survival rates, ranging from 68.62 ± 3.64% (FS46 strain) to 100.89 ± 3.52% (FS44 strain).

Table 3. Resistance of LAB strains to simulated gastrointestinal conditions.

	Survival Rate (%)			
Isolates	**pH 1.5**	**Pepsin (0.1%)/pH 2.5**	**Phenol (0.4%)**	**Bile Salts (0.3%)**
FS43	84.35 ± 0.85 ^{d}	91.58 ± 3.43 ^{def}	94.58 ± 3.70 ^{c}	82.25 ± 0.75 ^{c}
FS51	83.33 ± 0.91 ^{d}	89.98 ± 1.81 ^{ef}	40.59 ± 2.80 ^{g}	93.09 ± 1.25 ^{b}
FS50	87.05 ± 1.95 ^{c}	94.88 ± 2.41 ^{bcd}	44.12 ± 3.01 ^{fg}	99.58 ± 2.07 ^{a}
FS49	81.18 ± 1.05 ^{e}	91.45 ± 1.78 ^{def}	85.12 ± 6.60 ^{d}	79.63 ± 3.90 ^{cd}
FS65	98.15 ± 0.95 ^{a}	95.60 ± 0.84 ^{bc}	48.09 ± 2.80 ^{f}	77.791 ± 0.40 ^{de}
FS48	88.30 ± 0.91 ^{c}	99.78 ± 2.14 ^{a}	128.24 ± 1.08 ^{a}	77.49 ± 0.59 ^{de}
FS47	91.38 ± 0.88 ^{b}	92.63 ± 1.78 ^{cde}	112.64 ± 1.60 ^{b}	75.32 ± 2.15 ^{e}
FS46	90.74 ± 1.80 ^{b}	97.96 ± 0.84 ^{ab}	73.72 ± 0.78 ^{e}	68.62 ± 3.64 ^{f}
FS45	85.27 ± 0.57 ^{d}	92.44 ± 1.88 ^{cde}	90.22 ± 0.44 ^{cd}	74.18 ± 1.15 ^{e}
FS44	85.03 ± 0.98 ^{d}	88.54 ± 1.26 ^{f}	109.46 ± 0.51 ^{b}	100.89 ± 3.52 ^{a}

Each value represents the mean of three replicates, accompanied by their respective standard deviations. Values bearing the same lowercase letter (a, b, c, d, e, etc.) are not statistically significantly different at the 5% level within the same column.

3.3.2. Cell Surface Characteristics

The study of co-aggregation, auto-aggregation, and hydrophobicity offers insights into the adhesion and colonization behavior of LAB strains in the host intestinal tract. The co-aggregation rates of LAB strains with *S.* Typhimurium were significantly higher than those with *E. coli* (Figure 2). All LAB strains showed high co-aggregation rates (>55%)

against *S.* Typhimurium. In contrast, *E. coli* showed the lowest percent co-aggregation with FS47 (20.80 ± 1.22%) and the highest with FS46 (50.89 ± 1.00%). Additionally, the FS46 strain exhibited high co-aggregation with *S.* Typhimurium (71.78 ± 0.85%) (Figure 2).

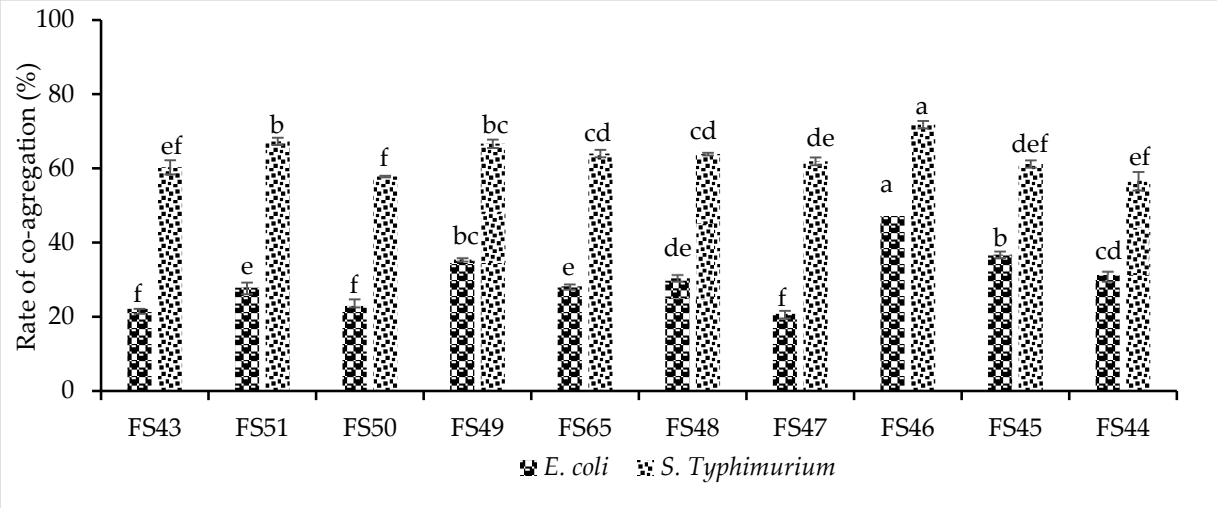

Figure 2. Co-aggregation rates of LAB strains with *E. coli* ATCC 8739 and *S.* Typhimurium ATCC 14028. Each value was expressed as the mean ± standard deviation (SD). Statistically significant differences ($p < 0.05$) were indicated by different lowercase letters above the error bars.

The functional ability of LAB strains to adhere to epithelial cells is closely linked to their auto-aggregation ability and cell surface hydrophobicity (Figure 3). The strains FS47, FS49, and FS44 demonstrated a relatively high hydrophobicity in xylene, exceeding 57%. Conversely, strain FS43 showed minimal hydrophobicity (35.23 ± 4.43%). All LAB strains demonstrated a significant level of auto-aggregation (>50%) after 24 h of incubation. Four LAB strains (FS47, FS48, FS45, and FS46) exhibited exceptionally high auto-aggregation, resulting in the formation of a visible precipitate at the bottom of the tubes.

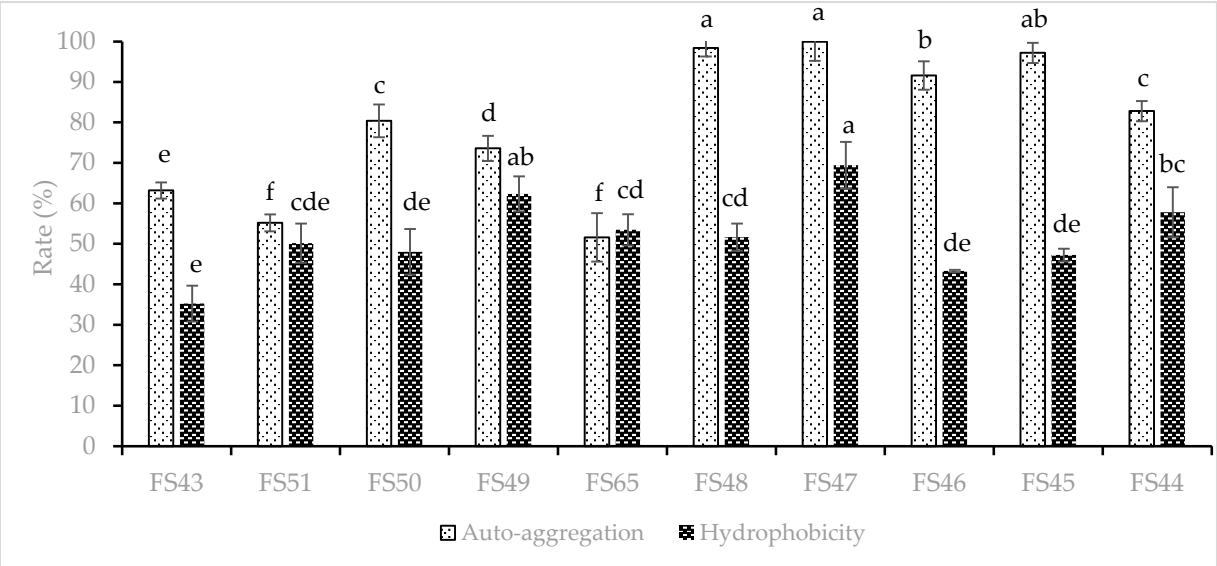

Figure 3. Auto-aggregation and hydrophobicity rates of LAB strains. Each value was expressed as the mean ± standard deviation (SD). Statistically significant differences ($p < 0.05$) were indicated by different lowercase letters above the error bars.

3.4. Exploration of Probiotic Properties

3.4.1. Antibacterial Spectrum

The antibacterial activity of the CFSs from the LAB strains against four pathogenic species is shown in Table 4. The LAB strains exhibited high antagonist activity against *S. aureus* ATCC 33592, *L. monocytogenes* ATCC 7644, and *S. enterica* serovar Typhimurium ATCC 14028, with inhibition diameters between 18 and 29 mm. Strains FS43, FS65, FS48, FS46, and FS44 demonstrated significant antibacterial activity against *E. coli* ATCC 8739. However, strains FS51, FS50, FS49, FS47, and FS45 demonstrated moderate antibacterial activity against *E. coli*, with inhibition diameters between 6 and 10 mm.

Table 4. Antimicrobial activity of LAB strains.

Isolates	*E. coli* ATCC 8739	*L. monocytogenes* ATCC 7644	*S. enterica* Serovar Typhimurium ATCC 14028	*S. aureus* ATCC 33592
FS43	+++	+++	+++	+++
FS51	++	+++	+++	+++
FS50	++	+++	+++	+++
FS49	++	+++	+++	+++
FS65	+++	+++	+++	+++
FS48	+++	+++	+++	+++
FS47	++	+++	+++	+++
FS46	+++	+++	+++	+++
FS45	++	+++	+++	+++
FS44	+++	+++	+++	+++

Legend: (-) the absence of a halo formation; (+) the presence of a halo measuring 1–5 mm in diameter; (++) the presence of a halo measuring 5–10 mm in diameter; (+++) the presence of a halo measuring >10 mm in diameter.

3.4.2. Antioxidant activity

The DPPH assay was used to evaluate the antioxidant capacity of both intact LAB cells and their supernatants (see Figure 4). Both the intact LAB cells and supernatants of the FS48 strain showed statistically significant antioxidant activity levels ($p < 0.05$). Except for the FS44 strain, the antioxidant activity levels detected in the intact cells were significantly higher than those in the free cell supernatants, as shown in Figure 4. Seven strains (FS46, FS45, FS49, FS43, FS65, FS47, and FS48) exhibited DPPH free radical scavenging rates ranging from 51.64% to 83.58%, while the remainder of strains showed rates below 50%. Overall, the CFS from strain FS65 showed the lowest antioxidant activity (13.87 ± 0.44%), while the CFS from strain FS48 showed the highest antioxidant activity (49.09 ± 0.88%).

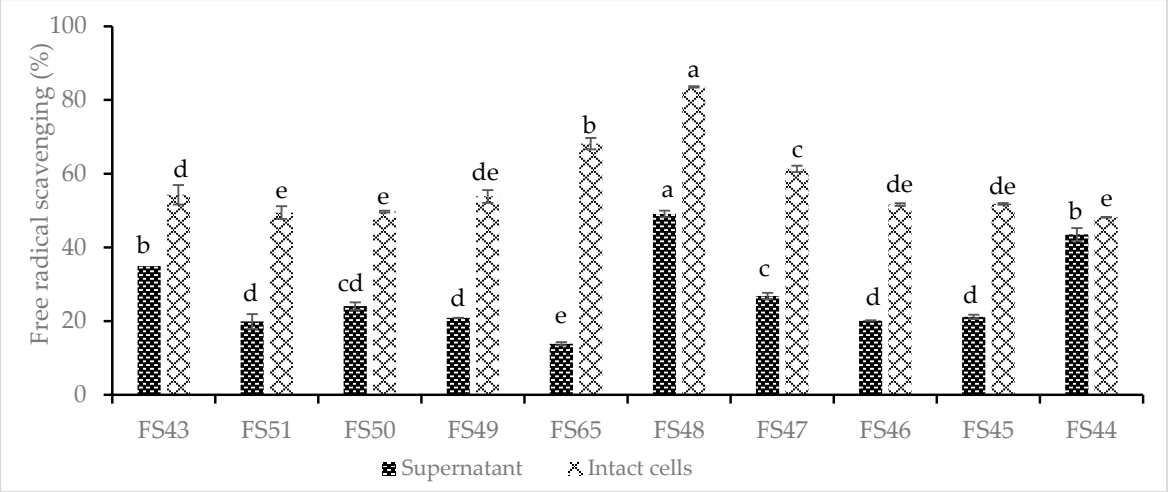

Figure 4. Antioxidant capacity of free cell supernatants and intact LAB cells evaluated via DPPH

assay. Each value was expressed as the mean ± standard deviation (SD). Statistically significant differences ($p < 0.05$) were indicated by different lowercase letters above the error bars.

3.4.3. Enzymatic Activity of LAB Strains

The LAB isolates were inoculated on selective media to detect amylase, cellulase, lipase, pectinase, and protease activities (refer to Table 5). The results showed that all LAB strains tested were positive for protease and cellulase activities. Out of the ten strains tested, only FS43 exhibited positive lipolytic activity. Eight LAB strains demonstrated positive pectinolytic activity. Furthermore, five strains (FS51, FS49, FS65, FS48, and FS45) showed positive amylase activity, while the others did not.

Table 5. Enzymatic activities of LAB strains.

Parameters / Strains	Enzymatic Activities				
	Proteolytic	**Lipolytic**	**Pectinolytic**	**Cellulolytic**	**Amylolytic**
FS43	+	+	+	+	-
FS51	+	-	+	+	+
FS50	+	-	+	+	-
FS49	+	-	-	+	+
FS65	+	-	+	+	+
FS48	+	-	-	+	+
FS47	+	-	+	+	-
FS46	+	-	+	+	-
FS45	+	-	+	+	+
FS44	+	-	+	+	-

The (+) symbol indicates that the enzymatic activity is positive, while the (-) symbol indicates that the enzymatic activity is negative.

3.5. Principal Component Analysis (PCA)

Principal component analysis (PCA) and hierarchical ascending classification (HAC) are powerful methods for synthesizing diverse information to enhance comprehension. The analysis results indicated that the combined influence of the two principal components (F1 and F2) explained 77.51% of the total variation. Of this, 65.03% was attributed to component F1 and 12.48% to component F2 (Figure 5). According to the PCA, based on the probiotic attributes of the LAB isolates, they could be divided into four distinct groups. The first group comprises strains FS43, FS44, and FS48 (positive correlation of both F1 and F2) and exhibited the highest values for hydrophobicity, phenol tolerance, antioxidant activity of the free cell supernatants, and intact cells (Figure 5). These three strains were classified as belonging to the same class based on the hierarchical ascending categorization of similarities (Figure 6). The second group consists of the FS50 and FS51 strains (positive side of F2 and negative side of F1) and exhibited high values for 0.3% bile salt tolerance. The third group comprises strains FS65, FS49, and FS46 (negative of both F1 and F2) and exhibited high values for pH 1.5 tolerance and co-aggregation ability with *S*. Typhimurium. In the fourth group, the strains FS45 and FS47 (positive side of F1 and negative side of F2, respectively) exhibited high values for pH 2.5 and 0.1% pepsin resistance, co-aggregation with *E. coli*, and auto-aggregation abilities, as well as a range of other probiotic properties. The results indicated that the strains FS43, FS44, and FS48 demonstrated the greatest probiotic potential.

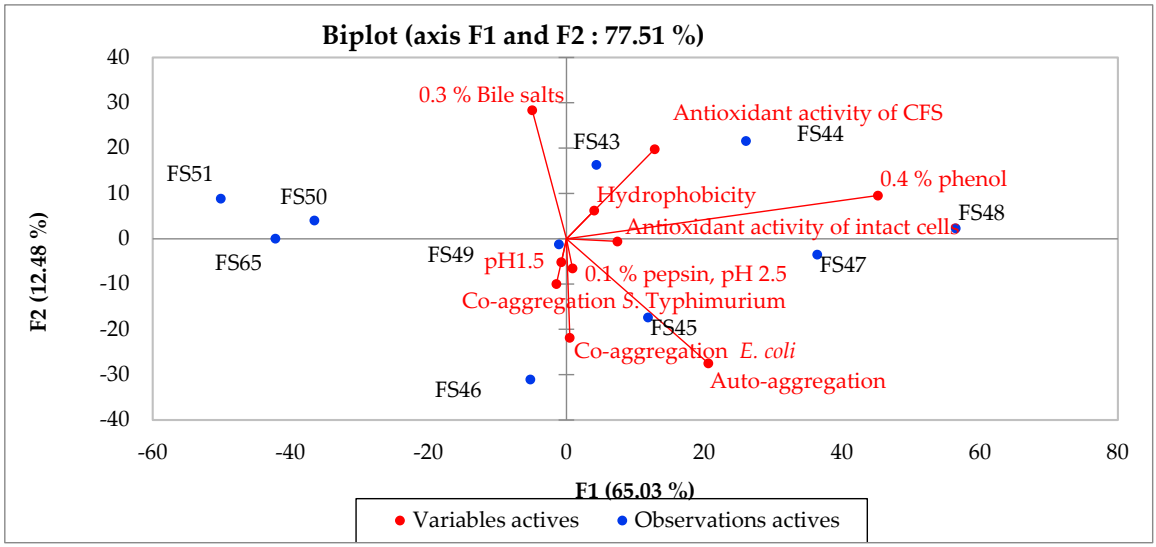

Figure 5. Principal component analysis of LAB strains according to probiotic properties.

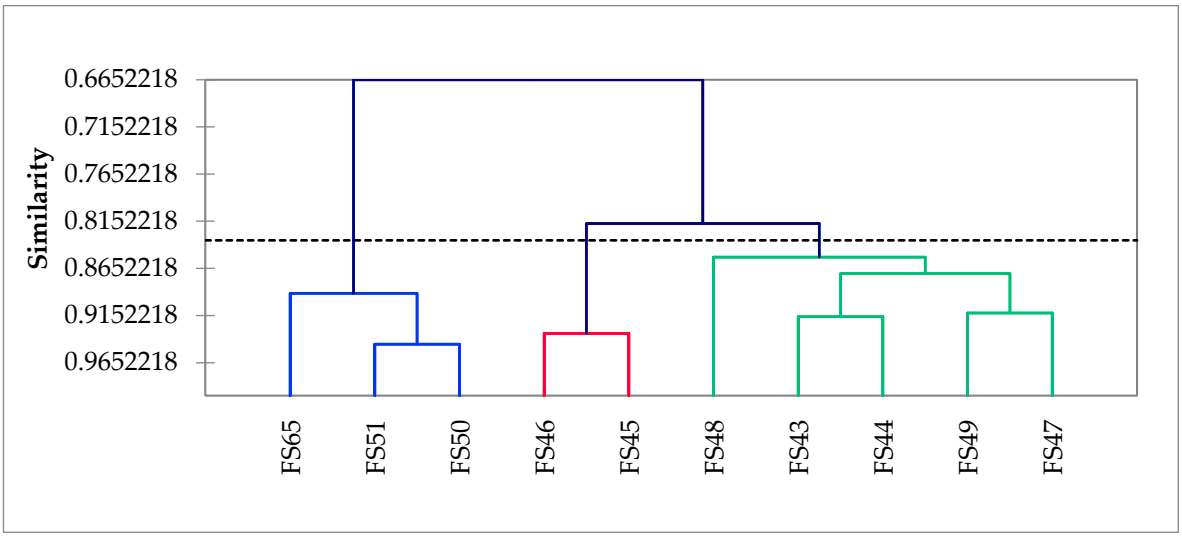

Figure 6. Hierarchical ascending classification of LAB strains according to probiotic properties. The dotted line delineates the cutoff point of this specific algorithm, which generates three clusters (indicated by distinct colors) based on probiotic characteristics that exhibit a high degree of similarity.

4. Discussion

Africa has a long history of producing traditional fermented foods in local dietary contexts, including those derived from cassava, maize, millet, tubers, wild legume seeds, and sorghum [6–9]. Additionally, fermented milk, meat products, and alcoholic beverages have been produced on the continent for centuries [6–9]. Given that a significant proportion of African foods are fermented by lactic acid bacteria, they can be isolated and studied for their multifunctional ability to ferment foods, impart distinct taste and high nutritional value, and ensure food safety over an extended period. Additionally, they can be studied for their probiotic properties, which could have beneficial health effects. This process also contributes to an enhanced health status in those who consume them, which is of great importance in the African context [8]. The review by Pereira et al. [26] provides a comprehensive overview of the criteria and the methodologies employed for the selection of probiotics, such as their ability to tolerate unfavorable conditions in the human digestive system, adhere to human body cells, exhibit antimicrobial and antioxidant

activities, synthesize enzyme, demonstrate antibiotic sensitivity, undergo hemolysis test, and fulfill other pertinent criteria.

This study aimed to assess the probiotic potential of 10 bacterial isolates obtained from the spontaneous fermentation of dockounou paste. The new isolates were preliminarily identified as lactic acid bacteria based on their colonial aspects (shape round or smooth), microscopic observations (bacilli, Gram-positive), and biochemical characteristics (negative oxidase and catalase). All LAB strains were identified as *L. plantarum* based on the high degree of homology observed between their 16S rDNA gene sequences and those deposited in the NCBI database. *Lactobacillus* spp. (particularly *L. plantarum* and *L. fermentum)* have been predominantly detected in various African fermented foods such as *doklu* (Ivorian maize dough) [12], Nigerian fermented foods [45–47], Maasai traditional fermented milk products [48], *ben-saalga* (Burkinabé fermented cereal gruel) [49]; *bushera* (Ugandan fermented cereal beverage) [50], *tchapalo* produced in Ivory Coast [51].

The paramount concern regarding probiotics must be their safety, which must not harm consumer health. The absence of hemolytic activity indicated that our LAB strains are unable to degrade blood constituents and, therefore, pose no pathological risk. Coulibaly et al. [28] also reported that *L. plantarum* and two other *Pediococcus* sp. isolated from the gut of Tilapia showed no hemolytic activity. The EFSA suggests that all microbial strains used as food or feed additives or probiotics should be subjected to testing to determine their sensitivity to different antibiotics [52]. Our findings have revealed that the majority of LAB strains demonstrated high or intermediate susceptibility to at least one antibiotic from the beta-lactam class (which inhibits cell wall synthesis) and the macrolide class (which inhibits protein synthesis). Furthermore, all strains demonstrated sensitivity to chloramphenicol, nitrofurans, and sulfonamide–diaminopyrimidine complex antibiotics. The overuse of antibiotics has resulted in the evolution and prevalence of resistant bacteria. Probiotics isolated from various dietary supplements or foods have demonstrated the ability to resist the effects of antibiotics [53–56]. Horizontal transfer of antibiotic-resistance genes from these probiotics to pathogenic bacteria residing in the intestinal tract could have significant health implications [54,55].

One of the key considerations when selecting LAB isolates as probiotics is their ability to withstand the challenges of the gastrointestinal tract. In our study, all LAB strains exhibited high survival rates, ranging between 81.18% and 91.38% in an acidic environment with a pH of 1.5. The LAB strains also demonstrated high survival rates, between 88.54% and 99.78%, in the presence of pepsin (0.1%) at a pH of 2.5. Furthermore, they exhibited high survival rates in the presence of bile salts (0.3%), ranging from 68.62 to 100.89%. Various strains of lactic acid bacteria demonstrated high survival rates, indicating their ability to withstand the acidic environment of the stomach (pH 1.5–3.0) and the small intestine (bile salts concentrations of 0.1–0.3%) [28,36,57]. Likewise, *Lactobacillus* spp. isolated from Shamita and Kocho (traditional Ethiopian beverages and foods) demonstrated that they can survive in extremely acidic conditions (greater than 80%) for 6 h [58]. Two *L. plantarum* strains, isolated from West African fermented cereals, exhibited comparable levels of acid resistance (pH 2.0) and tolerance to a bile salt concentration of 0.3% [47]. *L. plantarum* strains, isolated from traditional fermented milk of the Maasai in Kenya, demonstrated acid resistance at pH 2.0, with survival rates varying from 1% to 100% [48]. In a relatively recent study, Matei et al. [59] found that three strains of *Pediococcus pentosaceus* showed strong tolerance to high concentrations of bile salts (3% and 6%). It is widely accepted that the capacity of bacteria to resist phenol is an important indicator of their general viability. It has been observed that some bacterial species can synthesize harmful metabolites that can be deaminated by gut bacteria, creating toxic compounds with bacteriostatic properties such as phenol. Our results revealed that certain *L. plantarum* isolates exhibited greater resistance to phenol (0.4%) (73.72–128.24% viability) compared to others. For instance, *L. plantarum* strains varied in their ability to resist phenol, which had different effects on them [60]. *Lactobacillus* strains derived from traditional fermented foods, multigrain millet dosa batter, exhibited resistance to 0.2–0.8% phenols, maintaining viability at 53.3%–83.6% [61]. Fur-

thermore, in our study, three *L. plantarum* isolates demonstrated not only high resistance but also were able to grow in the presence of 0.4% phenol.

The interaction between microorganisms and epithelial cells is influenced by cell wall hydrophobicity [62–64]. This parameter can be employed to determine the adhesion capability of lactic acid bacteria to the solvent. High hydrophobicity indicates a strong interaction between LAB isolates and epithelial cells, resulting in enhanced pathogen exclusion. It is generally recommended to select a strain with a hydrophobicity of over 40% as a probiotic. The present study demonstrated that eight LAB strains exhibited hydrophobicity levels ranging from 47.23% to 69.43%. The results of this study are consistent with those of Yasmin et al. [65], who observed that *Bifidobacterium* strains exhibited high hydrophobicity when exposed to xylene. However, strains of *P. pentosaceus* and *L. plantarum* isolated from the gut of Tilapia exhibited a range of cell surface hydrophobicity in the presence of different organic solvents such as chloroform (9.4–87.2%), xylene (3.48–51.10%), and hexane (1.53–16.30%) [28]. This study demonstrated that *L. plantarum* strains exhibited a high degree of auto-aggregation (>50% at 24 h), indicating that they exhibited an adequate level of adherence, which is in agreement with previously reported results [65]. Also, the co-aggregation rate of LAB isolates was notably high with *S*. Typhimurium (57.80–71.78%), followed by *E. coli* (20.80–50.89%). Honey and Keerthi [66] observed that *L. plantarum* strains exhibited excellent auto-aggregation ability, with values ranging from 99.2% to 99.8%. The highest levels of co-aggregation with *S. typhi* were also noted, with values between 45.3% and 63.66% [66].

Lactic acid bacteria are capable of suppressing the growth of pathogenic microorganisms through the synthesis of antimicrobial metabolites (e.g., short-chain fatty acids, bacteriocins, hydrogen peroxide (H_2O_2), and others) and by competing with those for nutrients, as well as by adhering to epithelial cells [67,68]. The current study found that all LAB isolates demonstrated strong inhibitory effects against *S. aureus*, *L. monocytogenes*, and *S. enterica* serovar Typhimurium, with moderate effects against *E. coli*. These findings are consistent with prior research indicating the antagonist activity of *Lactobacillus* and *Pediococcus* strains against a wide range of bacterial pathogens, including those belonging to the Gram-negative and Gram-positive categories, which may contaminate food sources and cause intestinal illnesses [28,36,68–70].

Lactic acid bacteria have been extensively studied for their potential as probiotics, given their well-documented health benefits, which appear to be partially attributed to their antioxidant traits [71,72]. In the current study, most of the intact LAB cells exhibited high antioxidant activity, demonstrated by a high percentage reduction in DPPH with an average of 52% to 84%. The present study revealed a significant increase in antioxidant activity in intact cells compared to free-cell supernatants. These results contrast with those reported by Coulibaly et al. [28].

Lactobacilli have been identified as producers of enzyme complexes that enhance the digestibility of food and feed, as well as the activities of digestive enzymes and promote growth performance [73]. The evaluation of probiotic-producing enzymes can be conducted by cultivating candidate strains in culture media supplemented with precursors. These include casein, starch, carboxymethylcellulose, Tween 80 or olive oil, and sodium phytate, which are used to assess the activity of protease, amylase, cellulase, lipases, and phytases, respectively. The results of our study indicated that all LAB isolates possessed proteolytic and cellulolytic activities, with some also exhibiting pectinolytic or amylolytic activities. This result differs from the findings of Coulibaly et al. [28], in which the *P. pentosaceus* and *L. plantarum* strains isolated from the gut of Tilapia demonstrated lipase and β-galactosidase activity (with a few exceptions) but lacked amylase, cellulase, and protease activities. *L. fermentum* URLP18 isolated from *C. mrigala* demonstrated a high capacity for extracellular enzyme production, including amylase, protease, and lipase [74].

This study's results indicate that the isolation of lactic acid bacteria from naturally fermented and indigenous products is a topic worthy of further investigation. Nevertheless, strains FS43, FS44, and FS48 were identified as the most promising candidates for further

investigation, as they exhibited the greatest potential for beneficial effects, as indicated by principal component analysis (PCA) and hierarchical ascending classification (HAC). Artisanal fermented products are widely produced and consumed in traditional households and can be considered a valuable source of probiotics for improving the health of the Ivorian population.

5. Conclusions

The 10 lactic acid bacteria isolated from fermented plantain dockounou paste have been identified through 16S rDNA sequence analysis as *Lactiplantibacillus plantarum*. All LAB strains demonstrated high survival rate against pepsin (0.1%) and low pH (1.5 and 2.5) and exhibited high tolerance to bile salts (0.3%) and phenol (0.4%). Furthermore, all strains demonstrated high antioxidant activity and broad antibacterial activity against the main foodborne pathogens. The obtained findings indicated that three of the 10 LAB strains exhibited a high affinity towards xylene (more than 57%). All LAB strains exhibited high auto-aggregation properties, as well as strong degrees of co-aggregation with *S. enterica* serovar Typhimurium, followed by *E. coli*. Furthermore, the LAB isolates demonstrated no hemolytic activity and sensitivity to different antibiotic classes. The LAB isolates have been detected as valuable producers of bioactive multi-enzymes. Strains FS43, FS44, and FS48 exhibited multifunctional properties, suggesting that further *in vivo* research is required to assess their suitability as probiotics or for the development of innovative functional foods.

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