https://doi.org/10.31925/farmacia.2026.3.9 **ORIGINAL ARTICLE**

FUNCTIONAL PROPERTIES, VIABILITY AND ADHESION CAPACITY OF PROBIOTIC LACTIC ACID BACTERIA CULTIVATED IN *ALOE VERA* - SUPPLEMENTED CULTURE MEDIA

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Abstract

This study investigated the fermentative behaviour and functional properties of probiotic lactic acid bacteria cultivated in culture media supplemented with *Aloe vera*. Three probiotic strains – *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus* GG and *Lacticaseibacillus casei* – were grown in MRS medium and MRS supplemented with 0.5% (w/v) lyophilized *Aloe vera* powder. Fermentation dynamics were monitored through pH evolution and viable cell counts during 72 h of cultivation and during 21 days of refrigerated storage. *Aloe vera* supplementation supported both the growth and survival of probiotic bacteria during fermentation and subsequent cold storage, allowing viable counts to remain above the recommended probiotic threshold. The tested strains, *L. plantarum* and *L. casei* adapted most successfully to the supplemented medium and were subsequently evaluated for their ability to adhere to epithelial cells. Both strains adhered to the HeLa-2 cell line and reduced the attachment of pathogens such as *Staphylococcus aureus* and *Candida albicans*. The combined use of probiotic lactic acid bacteria and Aloe vera may provide a valuable basis for the development of functional fermented products with potential health benefits.

Rezumat

Acest studiu a investigat comportamentul fermentativ și proprietățile funcționale ale bacteriilor lactice probiotice cultivate în medii de cultură suplimentate cu *Aloe vera*. Trei tulpini probiotice – *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus* GG și *Lacticaseibacillus casei* – au fost cultivate în mediu MRS și în MRS suplimentat cu 0,5% (m/v) pulbere liofilizată de *Aloe vera*. Dinamica fermentației a fost monitorizată prin evoluția pH-ului și determinarea numărului de celule viabile pe parcursul a 72 de ore de cultivare și pe durata a 21 de zile de păstrare la temperatură de refrigerare. Suplimentarea mediului cu *Aloe vera* a susținut atât creșterea, cât și supraviețuirea bacteriilor probiotice în timpul fermentației și al depozitării la temperatură scăzută, menținând numărul de celule viabile peste pragul recomandat pentru produsele probiotice. Dintre tulpinile testate, *L. plantarum* și *L. casei* s-au adaptat cel mai bine la mediul suplimentat și au fost evaluate ulterior în ceea ce privește capacitatea lor de aderare la celulele epiteliale. Ambele tulpini au aderat la linia celulară HeLa-2 și au redus atașarea microorganismelor patogene, precum *Staphylococcus aureus* și *Candida albicans*. Utilizarea combinată a bacteriilor lactice probiotice și a *Aloe vera* poate oferi o bază valoroasă pentru dezvoltarea unor produse fermentate funcționale cu potențiale beneficii pentru sănătate.

Keywords: lactic acid bacteria, *Aloe vera*, probiotic fermentation, cell adhesion, antimicrobial activity

Introduction

Over the past decades, rapid economic and social development has been accompanied by an increasing prevalence of chronic health conditions, including cardiovascular diseases, metabolic disorders, intestinal dysfunctions, diabetes and various types of cancer. In this context, growing attention has been directed toward preventive strategies based on diet and functional foods. Among these, probiotic products have gained considerable interest due to their potential role in maintaining and improving human health, with reported benefits ranging from gut health and metabolic balance to immune regulation and mental well-being (1). Recent studies have highlighted the importance of probiotics in supporting

digestive health and overall well-being, which has contributed to a steady increase in consumer interest. In Europe, awareness of the role of the gut microbiome in health maintenance has significantly increased, leading to a higher demand for probiotic-based foods and dietary supplements. Consequently, the European probiotics market has shown substantial growth, being estimated at USD 14.26 billion in 2025 and projected to reach USD 16.93 billion by 2030 (2-4).

Probiotics are live microorganisms that, when administered in adequate amounts, confer a benefit to the host. Lactic acid bacteria (LAB) are a major group, frequently associated with traditional fermented foods, and LAB and yeast-based products contribute to the maintenance of a healthy intestinal environment (5). LAB exhibit specific morphological, physiological and metabolic characteristics and are naturally present on the mucosa of healthy individuals (6). In commercial products, the genera *Lactobacillus, Bifidobacterium, Enterococcus and Lactococcus* predominate (7). Moreover, species such as *Lacticaseibacillus casei* and *Lactiplantibacillus plantarum* are used as starter, adjuvant or protective cultures in food fermentation (8), while *Lacticaseibacillus rhamnosus*, *Limosilactobacillus frumenti* and *Limosilactobacillus reuteri* contribute to host health by modulating immunity, strengthening the epithelial barrier and inhibiting pathogens, supporting the balance of the microbiota (9). In addition, LAB can biotransform bioactive compounds during fermentation, for example, polyphenols are converted into simpler phenolic compounds with increased bioactivity, influencing their concentration and biological activity, including their antioxidant potential (10).

In recent years, consumer preference for natural, minimally processed, plant-based foods have increased, leading to the use of plant substrates in the development of functional fermented products (11). Numerous fermented beverages derived from plant-based raw materials have been studied, including fruits such as oranges, apples, passion fruit, pomegranates, carrots, and lemons (12-14), and recently *Aloe vera* has been explored as a fermentation substrate (11, 15). *Aloe barbadensis* var. Miller (*Aloe vera*), a plant native to Africa, is recognized for its therapeutic properties (16, 17) and contains over 200 bioactive compounds (vitamins, minerals, polysaccharides, amino acids, phenolic compounds, anthraquinones, enzymes, saponins, phytosterols, and salicylic acid derivatives) (18). Due to this composition, it is used in the treatment of wounds, inflammation, and pathologies such as microbial infections, ulcers, diabetes, and certain types of cancer (19, 20). At the same time, it has a prebiotic potential, stimulating beneficial microorganisms, which supports the development of fermented *Aloe vera*-based products as an attractive option for consumers and food industry.

The present study aimed to investigate the fermentative and physiological behaviour of the probiotic strains *L. plantarum*, *L. rhamnosus* and *L. casei*, cultivated in media supplemented with *Aloe vera*. Following the evaluation of the fermentative performance and viability, the strains with superior results were subjected to additional analyses regarding probiotic functionality, in particular the ability to adhere to epithelial cells and the potential to inhibit the adhesion of pathogenic microorganisms. As a result, *L. plantarum* and *L. casei* were further investigated in terms of cell adhesion and competitive inhibition of pathogens, such as *Staphylococcus aureus* (ATCC 25922 and clinical isolates) and *Candida albicans* (ATCC 10231 and clinical isolates).

Materials and Methods

Obtaining and preservation of probiotic bacterial cultures

L. rhamnosus GG (Gyniel plus®, Hyllan Pharma, Romania), *L. plantarum* (BioAliment Platform collection, Faculty of Food Science and Engineering, Galați, Romania) and *L. casei* (GynOphylus®, Beaufour Ipsen Industries, United Kingdom) were isolated by successive subculturing on MRS agar medium. Bacterial suspensions were prepared from commercial products in sterile physiological saline (0.9% NaCl) and inoculated at a concentration of 2% (v/v) into liquid MRS medium. Cultures were incubated at 37°C for 24 h. Subcultures were further performed on MRS agar supplemented with 1% $CaCO_3$, and single colonies obtained after 48 h at 37°C were evaluated morphologically (macro- and microscopically). The isolates obtained from the commercial probiotic formula Gyniel plus® were further identified using the API 50 CHL system (bioMérieux, France). Carbohydrate fermentation profiles were determined according to the manufacturer's instructions and analysed using the APIweb™ database. The biochemical profile corresponded to *L. rhamnosus*, allowing its differentiation from the other probiotic species present in the commercial formulation. Only the confirmed *L. rhamnosus* isolate was used in the subsequent experiments. Pure cultures were propagated in MRS broth for 48 h at 37°C and preserved in 20% glycerol at low temperature. Prior to experimental use, LAB strains were activated by three successive passages (3% inoculum) and anaerobically cultured (AnaeroGen system) in MRS broth at 37°C for 24 h.

Plant material and preparation of freeze - dried Aloe vera powder

Leaves of *Aloe vera* plants (4 years old) were obtained from a greenhouse in Galați County, Romania. After washing with distilled water, the gel and cortical

parts were separated and subjected to lyophilization using a Martin Christ Alpha 1-4 LD plus freeze dryer. Prior to freeze-drying, the gel was stored at -18°C for 1 h. The resulting lyophilized powders were packed in glass containers, protected from light, hermetically sealed and stored at 4°C. Before use, the powder was sterilized under UV light for 30 min.

Inoculum preparation and standardization

Frozen stock cultures were rapidly thawed in a water bath at 45°C for 2 - 3 min. A volume of 1 mL was inoculated into 5 mL MRS broth and incubated anaerobically at 37°C for 48 h to obtain the primary inoculum. The inoculum was standardized by adjusting the optical density to OD_{600} = 0.5 using a UV/VIS 6505 Jenway spectrophotometer, by dilution or concentration with MRS medium.

Cultivation of probiotic bacteria

Two fermentation media were used: MRS broth (control) and MRS supplemented with 0.5% (w/v) lyophilized *Aloe vera* powder. Fermentations were carried out in 100 mL Erlenmeyer flasks containing 50 mL medium, inoculated with 2% (v/v) primary inoculum and incubated at 37°C for 72 h under stationary conditions (Binder BF 4000 incubator). Samples were collected at 24, 48 and 72 h for physicochemical and microbiological analyses under aseptic conditions. After fermentation, samples were stored at 4°C for 21 days and analysed after 7, 14 and 21 days. All experiments were performed in triplicate.

Aloe vera ethanolic extract

3.0605 g of lyophilized powder from the gel and cortex of a whole *Aloe vera* leaf, weighed on a high-precision analytical balance XS403SM Mettler-Toledo are subjected to grinding. The lyophilized powder is treated with successive amounts of laboratory reagent acetone (Chemical Co.), decanted, and the supernatant is removed to dechlorophyllize the extract. This operation is repeated 5 - 6 times until the extract acquires a yellowish colour. The remaining solvent is completely evaporated, the dry mass of lyophilized and dechlorophyllized *Aloe vera* powder is weighed. The mass of the vegetable powder is taken up with 25.6 g of 96% analytical reagent ethyl alcohol (Chemical Co.), to obtain a 10% alcoholic extract of lyophilized *Aloe vera*.

Analytical methods

pH determination

The pH was measured using a SevenEasy multiparameter pH meter type F20K (Mettler Toledo).

Determination of viable cell counts

Viable cell counts were determined by serial decimal dilution in 0.1% (w/v) peptone water (Merck), followed by plating on MRS agar (Merck). Plates were incubated anaerobically (Anaerocult® A, Merck) at 37°C for 48 h. All determinations were performed in triplicate.

Kinetic parameters of bacterial growth

The multiplication of probiotic strains during fermentation (0 - 72 h) was evaluated by determining: number of generations (n) according to the formula:

$$n = \frac{\log N - \log N0}{\log 2},$$

where, N0 – initial number of CFU/mL, N – number of CFU/mL after 24 h of cultivation; multiplication rate according to the formula:

$$v = \frac{n}{\tau}\ [\frac{1}{h}],$$

where, n – number of generations, τ – cultivation time (hours); and generation time (t) according to the formula:

$$t = \frac{1}{v}[h].$$

The degree of viability maintenance was expressed as the percentage of viable cells at different time intervals relative to the initial value.

Adhesion capacity of probiotic bacteria

Selection of strains and rationale for adhesion experiments

Based on the results obtained during the fermentation and storage studies, *L. casei* and *L. plantarum* exhibited the highest adaptability, growth performance and viability in *Aloe vera* supplemented media. Therefore, these two strains were selected for further evaluation of probiotic functionality through adhesion assays. In addition to the MRS medium used during the fermentation experiments, adhesion tests were performed using an electrolyte medium (EM) and media supplemented with *Aloe vera* alcoholic extract (AAE). These additional conditions were included to investigate whether different *Aloe vera* derived preparations and cultivation environments could influence bacterial adhesion to epithelial cells and the competitive exclusion of pathogenic microorganisms. The sample codes presented in Table I were assigned solely for the adhesion experiments to distinguish the different combinations of bacterial strains, culture media and *Aloe vera* supplementation.

Preparation of epithelial cell monolayer and adhesion assay

Since adhesion to epithelial surfaces is considered a key characteristic of probiotic microorganisms, their adhesion capacity was investigated using the HeLa-2 epithelial cell line as an in vitro model. This approach allowed the evaluation of bacterial interaction with epithelial cells and the potential competitive exclusion of pathogenic microorganisms. The HeLa-2 cell line (human cervical carcinoma) was used to evaluate the adhesion capacity of probiotic strains. Cells were maintained in Eagle's Minimum Essential Medium (EMEM, Gibco, pH = 7.6) and incubated at 37°C in a controlled atmosphere (95% air, 5% CO_2) until reaching 80 - 90% confluence. Cells were then trypsinised, centrifuged (1000 rpm, 2 min), and resuspended in fresh EMEM. The culture

medium was replaced every 2 days. LAB suspensions were standardized to OD_{600} = 0.5 (~ 1.5 × 10^8 CFU/mL). Twelve experimental variants were prepared using MRS medium, MRS supplemented with 2 mL of 10% *Aloe vera* alcoholic extract (AAE), and electrolyte-based medium (EM). The experimental design and coding of samples are presented in Table I.

Table I

Experimental variants and culture conditions used for adhesion assays

Code	Bacterial strain	Culture medium	Supplement
5	*L. casei*	MRS	–
7	*L. plantarum*	MRS	–
9	*L. casei* + *L. plantarum*	MRS	–
5'	*L. casei*	MRS	AAE
7'	*L. plantarum*	MRS	AAE
11	*L. casei* + *L. plantarum*	MRS	AAE
6	*L. casei*	EM	–
8	*L. plantarum*	EM	–
10	*L. casei* + *L. plantarum*	EM	–
6'	*L. casei*	EM	AAE
8'	*L. plantarum*	EM	AAE
12	*L. casei* + *L. plantarum*	EM	AAE

AAE – *Aloe vera* alcoholic extract; EM – electrolyte medium; MRS – de Man, Rogosa and Sharpe medium

The electrolyte-based medium had the following composition (mmol/L): NaCl 3.5; KCl 1.5; sodium citrate 2.9; D-glucose 20 (pH 6.2). LAB cells were harvested by centrifugation (6000 rpm, 15 min), washed three times with PBS and adjusted to McFarland 0.5 standard. Adhesion assays were performed according to the method described by Bouchard *et al*. (21). After co-incubation with HeLa-2 cells (2 h, 37°C), non-adherent bacteria were removed by washing with PBS (Phosphate Buffered Saline). Cells were fixed with methanol and stained with 10% Giemsa solution. Samples were examined under an Olympus BX 41 microscope (100x objective), and the adhesion index (AI) was calculated based on the proportion of epithelial cells with adhered bacteria.

Evaluation of anti-adhesion activity against pathogenic strains

The anti-adhesion capacity of fermented media was evaluated against the following indicator strains: *S. aureus* ATCC 25923, clinical *S. aureus* isolate (from skin wound), *C. albicans* ATCC 10231 and clinical *C. albicans* isolate (from vaginal exudate). LAB were cultivated in fermentation media for 48 h at 37°C (Binder BF 4000 incubator). The cultures were centrifuged at 6000 rpm for 10 min, and the supernatants were collected in sterile 15 mL Falcon tubes. Pathogenic strains were cultured in tryptic soy broth (TSB) for bacteria and yeast extract peptone-glycerol (YPG) medium for yeast, for 18 - 24 h at 37°C (*S. aureus*) and 25°C (*C. albicans*). Cell suspensions were prepared in PBS and adjusted to McFarland standards, corresponding to approximately 1.5 × 10^8 CFU/mL for bacterial strains and 3 × 10^8 CFU/mL for yeast. The anti-adhesion assay was performed using an adapted Cravioto method, according to Jankowska *et al*. (22), and following protocols described by Malik *et al*. (23) and Rizzo *et al*. (24). A 1:1 mixture of pathogen suspension and LAB supernatant was applied to HeLa-2 cell monolayers. After incubation, non-adherent bacteria were removed by washing with phosphate-buffered saline (PBS). The cell monolayers were fixed with methanol, stained with Giemsa solution, and examined microscopically using an Olympus BX 41 microscope (100× objective). For each sample, at least 100 epithelial cells were evaluated in randomly selected microscopic fields. The adhesion index (AI) was calculated as the percentage of epithelial cells showing adhered bacteria relative to the total number of examined cells. Adhesion percentage was considered the primary outcome measure of the assay.

Statistical analysis

Data processing (including cleaning, structuring, and preparation of the data set for statistical analysis) and graphic representations were performed in Microsoft Excel, version 16.89.1 (24091630 – Microsoft Corporation, Redmond, WA, USA). Statistical analysis was performed using SPSS software (version 20.0). All experiments were performed in triplicate, and each measurement was performed in duplicate. Results were expressed as mean ± standard deviation (SD), and 95% confidence intervals (95% CI) were calculated for the main variables of interest. Statistical analysis included one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for paired comparisons of means. Effect size was estimated using eta-squared (η^2) while the statistical significance was set at $p < 0.05$.

Results and Discussion

Dynamics of lactic fermentation and pH evolution

The dynamics of lactic fermentation were evaluated by monitoring pH changes in two culture media (MRS and MRS supplemented with 0.5% (w/v) lyophilized *Aloe vera* powder) for the probiotic strains *L. plantarum*, *L. rhamnosus* GG and *L. casei* during 72 h of fermentation and 21 days of refrigerated storage. A rapid decrease in pH was observed during the first 24 h of fermentation for all tested strains, indicating intense metabolic activity and organic acid production. For *L. rhamnosus* GG, the pH decreased from 5.84 to 4.04 in MRS medium and from 5.70 to 3.84 in *Aloe vera*-supplemented medium, without statistically significant differences between the two variants ($p > 0.05$), as shown in Figure 1. Both *L. plantarum* and *L. casei* showed a comparable fermentation profile in the tested media. The rapid pH decline observed during the first 24 h reflected intense metabolic activity

and organic acid production, whereas the subsequent stabilization suggested entry into the stationary phase. *L. plantarum* showed the most stable pH profile maintaining constant values from 24 h up to 21 days ($p < 0.05$), as illustrated in Figure 2, indicating good adaptability for both media.

A different trend was observed for *L. casei* which exhibited a slight increase in pH after 72 h, reaching approximately 4.23 in the medium enriched with *Aloe vera* (Figure 3). The slight increase in pH may reflect a gradual reduction in metabolic activity during storage.

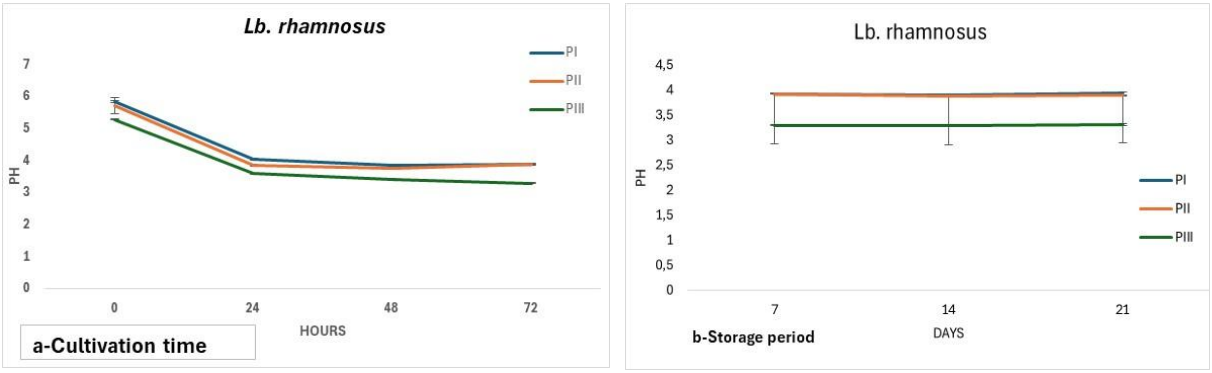

Figure 1.
pH variation of *L. rhamnosus* during 72 h of cultivation and 21 days of refrigerated storage in MRS medium and MRS supplemented with 0.5% (w/v) lyophilized *Aloe vera* powder
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

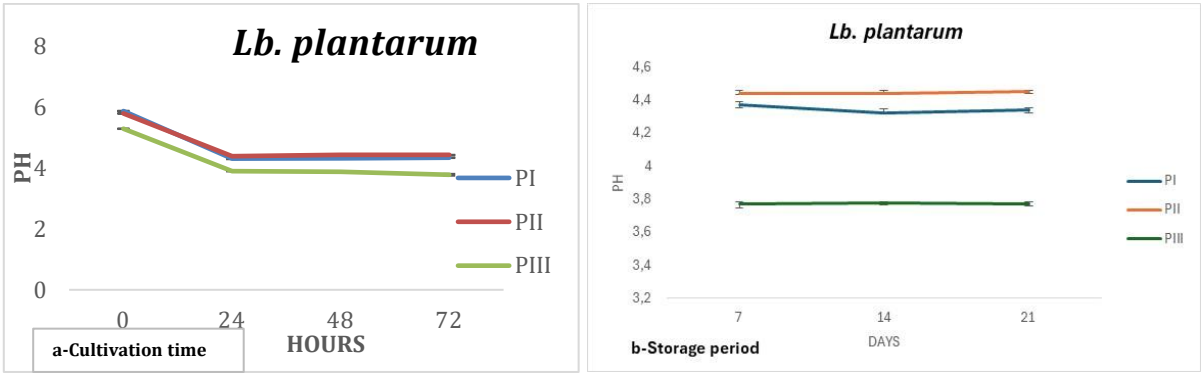

Figure 2.
pH variation of *L. plantarum* during cultivation and storage in control and Aloe vera-supplemented media
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

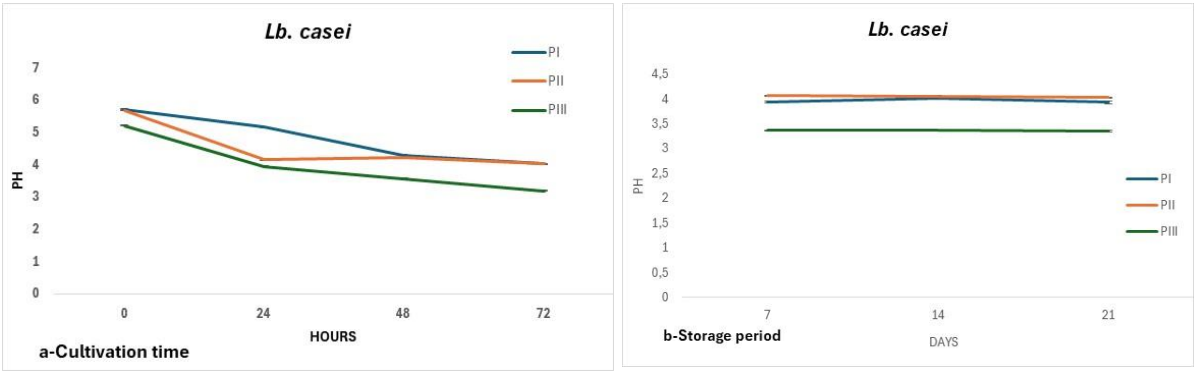

Figure 3.
pH variation of *L. casei* during cultivation and storage in control and *Aloe vera*-supplemented media
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

During refrigerated storage (4°C), pH values remained relatively stable for all samples, with only minor increases observed towards the end of the storage period, particularly in the control variants. The decrease in pH observed during fermentation is a direct consequence of the metabolic activity of lactic

acid bacteria, which leads to the accumulation of organic acids in the culture medium. This process may be further supported by the presence of prebiotic polysaccharides and other bioactive compounds in *Aloe vera*, which can stimulate bacterial growth and metabolic activity. All three strains (*L. plantarum, L. rhamnosus* GG and *L. casei*) were able to grow and survive under acidic conditions, demonstrating a successful adaptability in the fermentation environment. Acid tolerance is believed to be one of the major properties of probiotic bacteria as it is one of the factors contributing to their capacity to survive transit through the gastrointestinal track where pH can be as low as about 2 in the fasting state and around 4 in the presence of food (25). However, the ability to survive in acidic conditions may vary significantly among strains and strain-specific evaluation is required in the selection of suitable probiotic candidates (26, 27). The pH values obtained throughout fermentation and storage remained within the range considered suitable for probiotic products, confirming the acidification capacity of the investigated strains. The inclusion of *Aloe vera* did not interfere with the fermentation process and contributed to maintaining a stable environment for bacterial growth. This effect may be related to the presence of acemannan, glucomannan and other bioactive polysaccharides known to support LAB growth and metabolic activity, while also promoting the production of antimicrobial metabolites (15, 28-30). The current results are in line with earlier research indicating that Aloe vera based substrates are able to efficiently support LAB fermentation and maintain the pH values in the ideal range for probiotic products (3.5 - 4.7) (31-33). Taken together, all three strains showed good acidification capacity and adapted well to the tested media, whereas the presence of *Aloe vera* further supported fermentation.

Multiplication dynamics and viability during the fermentation period

The growth dynamics and viability of the probiotic strains were evaluated during 72 h of fermentation in MRS medium and MRS supplemented with 0.5% (w/v) lyophilized *Aloe vera* powder. For *L. rhamnosus* GG, the highest multiplication rate was observed during the first 24 h of fermentation, followed by a decrease between 24 and 48 h and a stabilization phase, thereafter, as shown in Figure 4, indicating the transition from exponential growth to stationary phase ($p < 0.05$). In the *Aloe vera*-supplemented medium, viable cell counts remained relatively constant up to 48 h, followed by a more pronounced decrease after 72 h.

In the case of *L. plantarum*, a similar growth pattern was observed in both media (Figure 5). However, slightly lower viable cell counts were recorded in the *Aloe vera*-supplemented medium after 48 and 72 h ($p < 0.05$), suggesting a moderate influence of the substrate on growth dynamics.

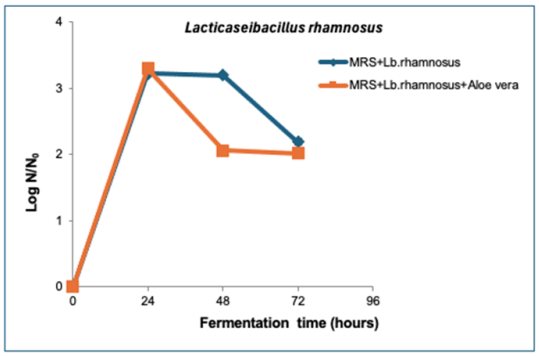

Figure 4.
Growth dynamics of *L. rhamnosus* during 72 h fermentation in MRS medium and MRS supplemented with 0.5% (w/v) *Aloe vera* powder
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

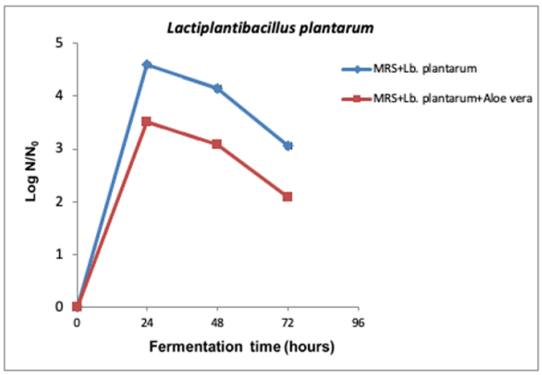

Figure 5.
Growth dynamics of *L. plantarum* during 72 h fermentation in MRS medium and MRS supplemented with 0.5% (w/v) *Aloe vera* powder
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

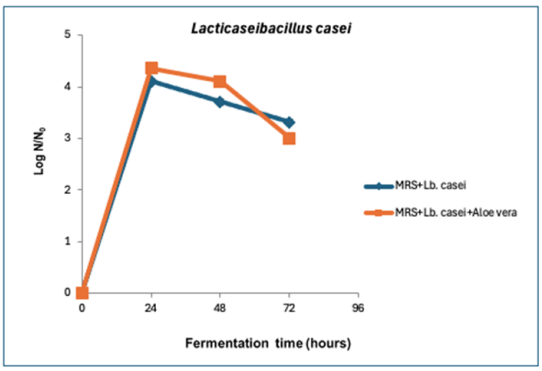

Figure 6.
Growth dynamics of *L. casei* during 72 h fermentation in MRS medium and MRS supplemented with 0.5% (w/v) *Aloe vera* powder
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

For *L. casei*, the maximum cell density was reached within the first 24 h of fermentation, particularly in the *Aloe vera*-supplemented medium, where values of 4.36 × 10^9 CFU/mL were recorded ($p < 0.05$). After 72 h, a gradual decrease in viable cell counts was observed in all variants, although higher values were maintained in the supplemented medium (3.01 × 10^8 CFU/mL), as illustrated in Figure 6.

Overall, all tested strains reached high viable cell densities, with values of approximately 8.0 log CFU/mL, indicating favourable growth conditions in both media. In all cases, the viable cell counts remained above the minimum level required for probiotic functionality (7.0 log CFU/mL). The kinetic parameters describing bacterial growth are presented in Table II. Values of kinetic parameters are expressed as mean SD of three experiments. Data bearing different lowercase letters (a–c) in the same compound are significantly different ($p < 0.05$).

Table II

Kinetic parameters of multiplication of probiotic bacteria (after 72 h of cultivation at 37°C)

Strain	Medium	Number of generations (n)	Multiplication rate (h^{-1})	Generation time (h)	Viability maintenance (%)
L. plantarum	MRS	13.82 ± 0.10[b]	0.57 ± 0.01[a]	1.75 ± 0.002[b]	71.10 ± 0.01[a]
	MRS + *Aloe vera*	16.77 ± 0.15[a]	0.69 ± 0.019[a]	1.43 ± 0.002[c]	61.56 ± 0.01[a]
***L. rhamnosus* GG**	MRS	10.66 ± 0.02[a]	0.44 ± 0.002[b]	2.27 ± 0.01[a]	73.98 ± 0.01[b]
	MRS + *Aloe vera*	10.96 ± 0.005[c]	0.45 ± 0.002[c]	2.19 ± 0.009[a]	69.23 ± 0.01[b]
L. casei	MRS	10.59 ± 0.01[a]	0.44 ± 0.002[b]	2.27 ± 0.02[a]	61.93 ± 0.03[c]
	MRS + *Aloe vera*	13.71 ± 0.01[b]	0.57 ± 0.004[b]	1.75 ± 0.004[b]	61.49 ± 0.02[a]

Aloe vera supplementation enhanced the growth performance of the tested strains, particularly *L. plantarum* and *L. casei*, which displayed higher numbers of generations and multiplication rates than in the control medium. In contrast, *L. rhamnosus* GG exhibited minimal changes across the two media. Generation time values were inversely correlated with multiplication rate, confirming faster growth under supplemented conditions. The degree of viability maintenance after 72 h ranged between approximately 61% and 74%, depending on the strain and culture conditions. Sustained growth and survival throughout fermentation demonstrated the adaptability of all tested strains. The supportive effect of *Aloe vera* can be attributed to its content of bioactive compounds and prebiotic polysaccharides, which may enhance microbial metabolism and proliferation.

The growth performance of the studied strains was comparable to that reported in previous studies evaluating LAB cultivation in *Aloe vera*-based substrates. Gonzales *et al*. (34) reported ultimate cell concentrations of approximately 1 × 10^9 CFU/mL for *L. plantarum* and 1 × 10^{10} CFU/mL for *L. casei*, together with higher multiplication rates than those observed in the present work. Such differences are probably connected to the composition of the fermentation substrate, since 0.5% of lyophilised *Aloe vera* powder was utilised in the present investigation, whereas full *Aloe vera* juice was used in the mentioned work. The kinetic parameters found in the present study are also comparable with those reported by Jyoti *et al*. (35) who observed a multiplication rate of about 0.38/h for *L. rhamnosus* cultivated in a citrate-glucose medium. Additionally, studies have previously demonstrated that changes in composition of the culture medium might have an impact on the growth and survival of bacteria. Supplementation with glucose alone or in combination with skimmed milk boosted the viable cell counts to 10^9 - 10^{10} CFU/mL after 48 h of incubation (36), levels similar to those seen in the present investigation. Of the strains examined, *L. casei* had the best kinetic profile in the *Aloe vera* supplemented medium. Its multiplication rate exceeded that reported by Rezvani *et al*. (37), suggesting a good ability of this strain to utilize the nutrients provided by the *Aloe vera* substrate. The favourable effect of *Aloe vera* on bacterial development may be related to its high content in polysaccharides and other bioactive compounds that can be used as substrates for probiotic microbes. Furthermore, *Aloe vera* combined with LAB may be considered as a potential symbiotic system. The interaction between *Aloe vera* constituents and probiotic bacteria may help to the suppression of harmful microorganisms and support the establishment of a balanced microbiota (38).

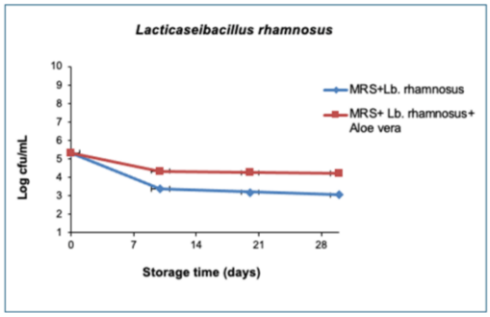

Figure 7.

Viability of *L. rhamnosus* during 21 days of refrigerated storage

Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

Viability of probiotic bacteria during the storage period

The fermented media were stored under refrigerated conditions (2 - 8°C) for 21 days, and the viability of

probiotic strains was evaluated after 7, 14 and 21 days. For *L. rhamnosus* GG, a decrease in viability was observed during the first 7 days of storage, followed by a slower decline up to 21 days ($p < 0.05$), as shown in Figure 7. A more pronounced reduction was recorded immediately after the transition from fermentation temperature (37°C) to refrigeration (4°C). However, the sample supplemented with *Aloe vera* exhibited a smaller decrease in viable cell counts and maintained higher CFU/mL values compared to the control.

In the case of *L. plantarum*, the highest viability was maintained throughout the storage period ($p < 0.05$). As illustrated in Figure 8, the *Aloe vera*-supplemented sample showed superior stability, with viable cell counts remaining above the recommended therapeutic level (6 log CFU/mL) even after 21 days. This result indicates a good adaptability of the strain and supports the role of *Aloe vera* as a favourable growth substrate.

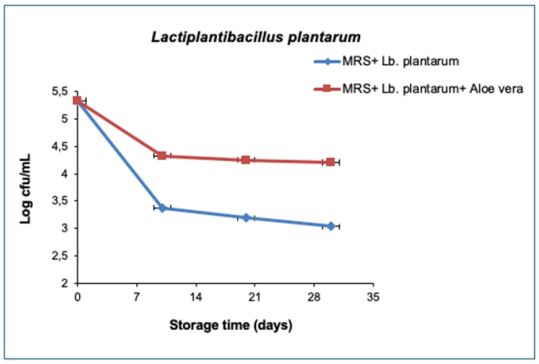

Figure 8.
Viability of *L. plantarum* during 21 days of refrigerated storage
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

For *L. casei*, the most significant decrease in viability was observed after 7 days of storage, particularly in the control sample. Subsequently, the rate of cell death decreased, especially in the *Aloe vera*-supplemented medium, indicating a protective effect of the plant matrix. After 21 days, the experimental sample maintained a viable cell count of approximately 5.63×10^7 CFU/mL ($p < 0.05$), as presented in Figure 9.

All the studied strains maintained viable cell counts above the minimum therapeutic threshold (6 log CFU/mL) during the storage period, especially in media supplemented with *Aloe vera*. The most stable strain was *L. plantarum*, next *L. casei* and *L. rhamnosus* GG was more sensitive to the storage conditions. The increased viability in the presence of *Aloe vera* could be due to the availability of dietary fibres and prebiotic chemicals that could help microbial survival during storage. Similar findings were reported in the literature with probiotic bacteria remaining viable for long periods under refrigerated conditions. For instance, prior investigations have shown that *L. casei* may survive up to 30 days at 2 - 8°C with ultimate concentrations of 5×10^6 CFU/mL (39). Other investigators have reported that *L. paracasei* strains persisted over the indicated therapeutic threshold during storage (40). The higher stability of *L. plantarum* and *L. casei* may also be associated with their stronger resilience to acidic environments and environmental stress. Besides, *Aloe vera's* prebiotic properties may improve metabolic activity and support bacterial life (41, 42). Makras *et al*. also reported that prebiotics can decrease the detrimental impacts of environmental stress on food matrices by supplying extra nutrients that increase the viability of probiotic bacteria (43). The synergistic interaction between the bioactive compounds of *Aloe vera* and lactic acid bacteria may further contribute to improved viability and functionality of probiotic systems. Such interactions can result in resistance to environmental stress and contribute to stability of probiotic populations during storage. Overall, *Aloe vera* exerted a protective effect on probiotic bacteria, helping to preserve viability during refrigerated storage. These observations also highlight the potential of *Aloe vera*-based systems for the development of stable functional fermented products.

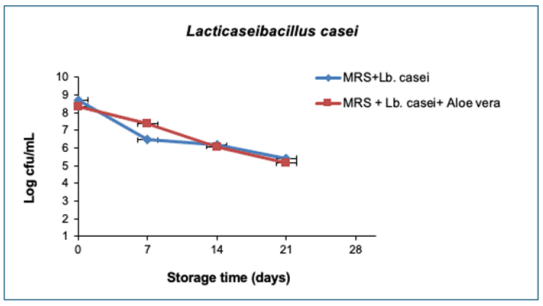

Figure 9.
Viability of *L. casei* during 21 days of refrigerated storage
Values are expressed as mean ± SD (n = 3). Error bars represent 95% confidence intervals

Adhesion capacity of probiotic lactic acid bacteria to the HeLa-2 cell line

The adhesion capacity of probiotic lactic acid bacteria (*L. casei* and *L. plantarum*) to the HeLa-2 epithelial cell line was evaluated under different cultivation conditions, including MRS medium, electrolyte medium (EM), and media supplemented with 2 mL of *Aloe vera* alcoholic extract (AAE). Adhesion was assessed microscopically following Giemsa staining and expressed as the adhesion index (AI, %), defined as the percentage of epithelial cells exhibiting adhered bacteria relative to the total number of examined cells.

The adhesion capacity varied according to the bacterial strain, culture medium, and presence of *Aloe*

vera extract. *L. casei* showed a significantly higher adhesion index in the presence of AAE (45%) compared with the non-supplemented control (30%) ($p < 0.05$). *L. plantarum* cultivated in AAE-supplemented medium exhibited a significantly higher adhesion index (70%) than the corresponding control cultivated in MRS medium (35%) ($p < 0.05$), representing a two-fold increase in adhesion capacity (Figure 10). These findings suggest that *Aloe vera*-derived bioactive compounds may enhance the adhesion potential of probiotic strains to epithelial cells.

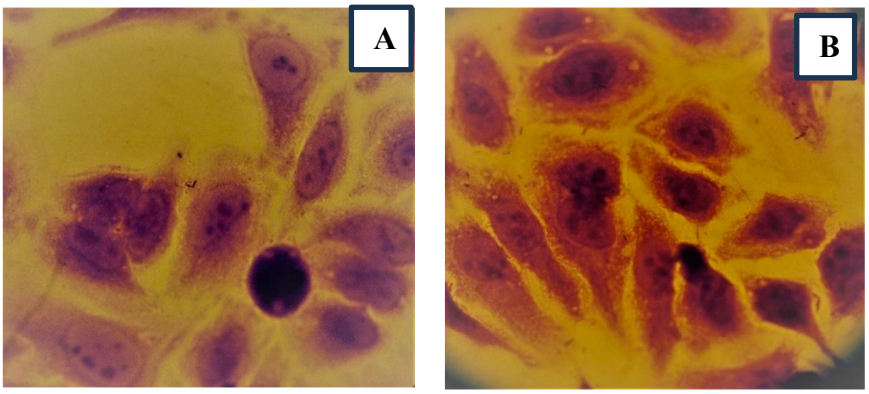

Figure 10.
Adhesion capacity of *L. casei* (A) and *L. plantarum* (B) to HeLa-2 epithelial cells after cultivation in MRS medium supplemented with *Aloe vera* alcoholic extract

In EM, the adhesion capacity of the probiotic strains was generally lower than that observed in MRS-based media. Nevertheless, supplementation with AAE markedly enhanced the adhesion of *L. plantarum*, increasing the adhesion index from 25% in the non-supplemented medium to 60% in the presence of the extract ($p < 0.05$), as shown in Figure 11. This outcome further supports a positive contribution of *Aloe vera*-derived compounds to the adhesive behaviour of *L. plantarum*.

The co-culture system supplemented with AAE achieved the highest adhesion index (75%), significantly exceeding the values observed for the individual cultures ($p < 0.05$), as presented in Figure 12. These observations indicate that *Aloe vera*-derived compounds may improve bacterial attachment to epithelial cells.

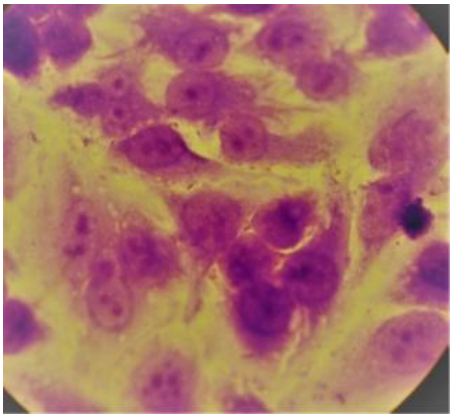

Figure 11.
Adhesion capacity of *L. plantarum* (sample 8′) to HeLa-2 epithelial cells after cultivation in MRS medium supplemented with Aloe vera alcoholic extract

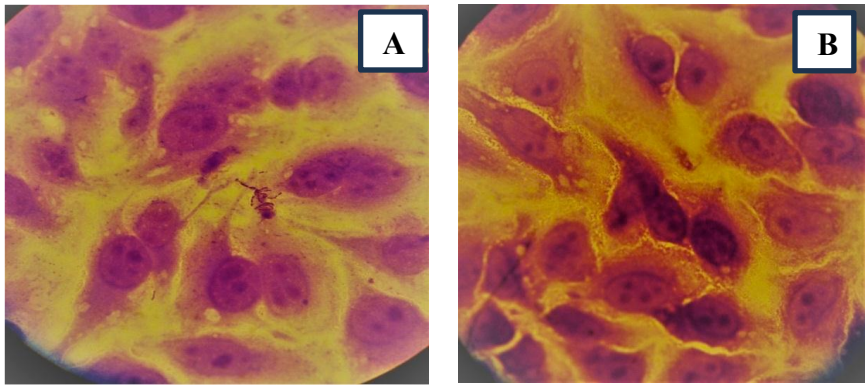

Figure 12.
Adhesion capacity of co-cultured *L. casei* and *L. plantarum* to HeLa-2 epithelial cells under different culture conditions (sample 11 – A; sample 12 – B)

The probiotic bacteria are characterised by their ability to adhere to epithelial cells thereby promoting colonisation and persistence in the host contributing to the suppression of pathogens. This is mediated *via* interactions between bacterial cell surface components and host epithelial receptors (44). In addition, adhesion capacity is highly correlated with auto-aggregation qualities and is an important factor for the prevention of pathogen colonisation and improvement of competitive exclusion (45, 46). The present study indicates that *Aloe vera* supplementation enhances the adhesive properties of probiotic strains probably due to its presence in bioactive components with antibacterial and prebiotic effects (47). To our knowledge, adherence of *L. plantarum, L. rhamnosus* GG and *L. casei* to the HeLa-2 cell line has not been described under these experimental conditions. The literature reports similar findings. For example, prior investigations have revealed the adherence of *Lactobacillus fermentum* to Caco-2 cells of around 4×10^7 CFU/mL (48). Furthermore, numerous LAB strains have shown a considerable ability to adhere and form biofilm on epithelial cell types, indicating their probiotic potential (49). In other cases, the adhesion capacity was even higher than that of known probiotic strains as *L. rhamnosus* GG (49). However, other studies have indicated that although LAB can adhere and form biofilms, they do not necessarily induce inflammatory responses in epithelial cells (50), highlighting their safety and suitability for functional applications. The adhesion of probiotic bacteria is generally transient but is associated with multiple beneficial effects, including modulation of the host microbiota and protection against pathogens (51). These findings demonstrate that both *L. casei* and *L. plantarum* exhibit good adhesion capacity, which is significantly enhanced by *Aloe vera* supplementation and co-cultivation. The results suggest the potential use of *Aloe vera*-based systems for the development of functional probiotic products with better colonization ability.

Anti-adhesion activity of pathogenic strains in probiotic fermented media

The anti-adhesion capacity of probiotic lactic acid bacteria was evaluated against pathogenic strains of *S. aureus* (ATCC and clinical isolate) and *Candida albicans* (ATCC and clinical isolate), using supernatants obtained from fermented media containing *L. casei* and *L. plantarum*, cultivated under different conditions.

The probiotic supernatants exerted a statistically significant inhibitory effect on pathogen adhesion to the HeLa-2 epithelial cell line. Inhibition rates ranged from 15% to 90%, depending on the probiotic strain, culture medium, and AAE supplementation. Significant differences were observed between experimental groups ($p < 0.05$), with the highest inhibition levels recorded for AAE-supplemented cultures, highlighting the contribution of *Aloe vera*-derived bioactive compounds to the anti-adhesion activity of the probiotic systems.

In the case of *S. aureus*, both individual and co-culture variants exhibited anti-adhesion activity, with the strongest effect observed in co-culture systems supplemented with AAE. Under these conditions, pathogen adhesion decreased from approximately 1.5×10^8 CFU/mL in the control to 1.5×10^7 CFU/mL. Individual cultures of *L. casei* showed a pronounced inhibitory effect, reducing adhesion by up to 85%, while *L. plantarum* exhibited a moderate but consistent activity. Microscopic observations confirmed these findings, revealing a clear reduction in bacterial attachment and significant modifications in the adhesion pattern, including loss of aggregation and irregular distribution on the epithelial surface, as illustrated in Figure 13 (A–D).

In the case of *Candida albicans*, probiotic strains also exhibited significant anti-adhesion activity. The strongest inhibitory effect was observed for *L. casei*, which reduced adhesion by up to 70%, both in individual and co-culture variants. The addition of AAE further enhanced this effect, particularly in MRS-based media, where pathogen counts decreased from approximately 3×10^8 CFU/mL to 6×10^7 CFU/mL. In contrast, *L. plantarum* showed a moderate antifungal effect, mainly in non-supplemented media, suggesting that the inhibition of *C. albicans* adhesion in co-culture systems is primarily associated with the activity of *L. casei*. Similar studies have been conducted to reveal the adhesion capacity of *Lb. plantarum* to HeLa-2 cell layer, and to measure competitive activity against *C. albicans* through specific suppression of IL-1, IL-6 and IL-8 (52). Microscopic analysis revealed marked alterations in fungal adhesion patterns, including reduced attachment and morphological changes, as shown in Figure 14 (A–D).

The anti-adhesion activity of probiotic bacteria can be explained by multiple mechanisms, including the production of organic acids (such as lactic and acetic acid), hydrogen peroxide and other antimicrobial compounds, as well as competitive exclusion and co-aggregation phenomena (53, 54). Research on *L. plantarum* and *L. casei* shows that they can exclude, compete with, and displace the tested *Candida spp*. strains, with a significant inhibition of pathogen adhesion ranging from 24.9% to 89.7% (55). Organic acids are known to be important in the inhibition of bacterial infections, but earlier research indicates that *Candida albicans* may be less susceptible to acidic conditions and its inhibition may be related to hydrogen peroxide production by lactobacilli (56-58). Furthermore, the observed change in adhesion patterns supports the idea of co-aggregation between probiotic and pathogenic microorganisms, which can limit pathogen attachment and colonisation (59). However, a growing number of studies demon-

strate that the adhesins of probiotic bacteria can function even independently of viable cells, and that bacterial viability is not always essential for exerting immunomodulatory effects (60). There has been a growing focus on mechanistic elements such as the involvement of adhesion in probiotic functionality to check if probiotics provide a potential health benefit (61). Adhesion to the intestinal mucosa enables LAB to compete with undesirable microorganisms for nutrients and colonization sites. This competitive exclusion helps to keep a balanced intestinal environment. The adhesion capabilities of LAB are mainly determined by the surface hydrophobicity, auto-aggregation and co-aggregation (54). For example, co-aggregation of LAB strains with other bacterial cells or mucus components can improve their adhesion to the intestinal epithelium or mucus layer. LAB may also interact with extracellular matrix molecules such as collagen, fibronectin and vitronectin that enhance adhesion (54). Besides, several investigations have revealed that certain probiotic bacterial strains exhibit anti-inflammatory effects by the stimulation of IL-10-dependent and TGF-β-mediated regulatory mechanisms contributing to immunological homeostasis (62). In conclusion, the results show that probiotic lactic acid bacteria, especially *L. casei* and *L. plantarum* combined with *Aloe vera*, have a considerable anti-adhesion effect against bacterial and fungal infections. The results demonstrate that probiotic fermentation systems are alternative techniques to avoid pathogen colonisation and promote host health.

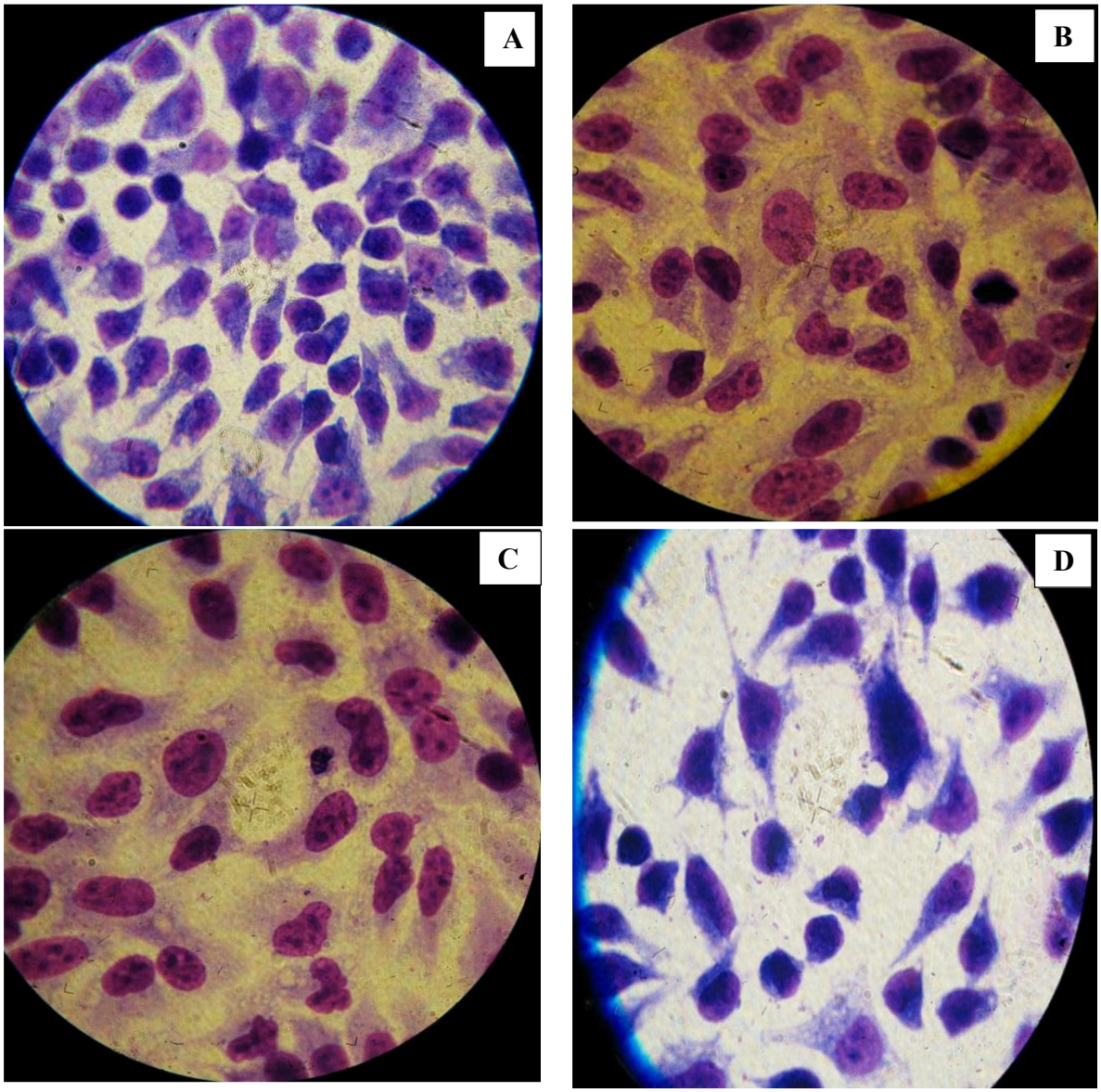

Figure 13.
Representative microscopic images showing the anti-adhesion effect of probiotic supernatants on *S. aureus* strains: (A) control; (B) *L. casei* (sample 5′); (C) *L. plantarum* (sample 7′); (D) co-culture (*L. casei* : *L. plantarum*)

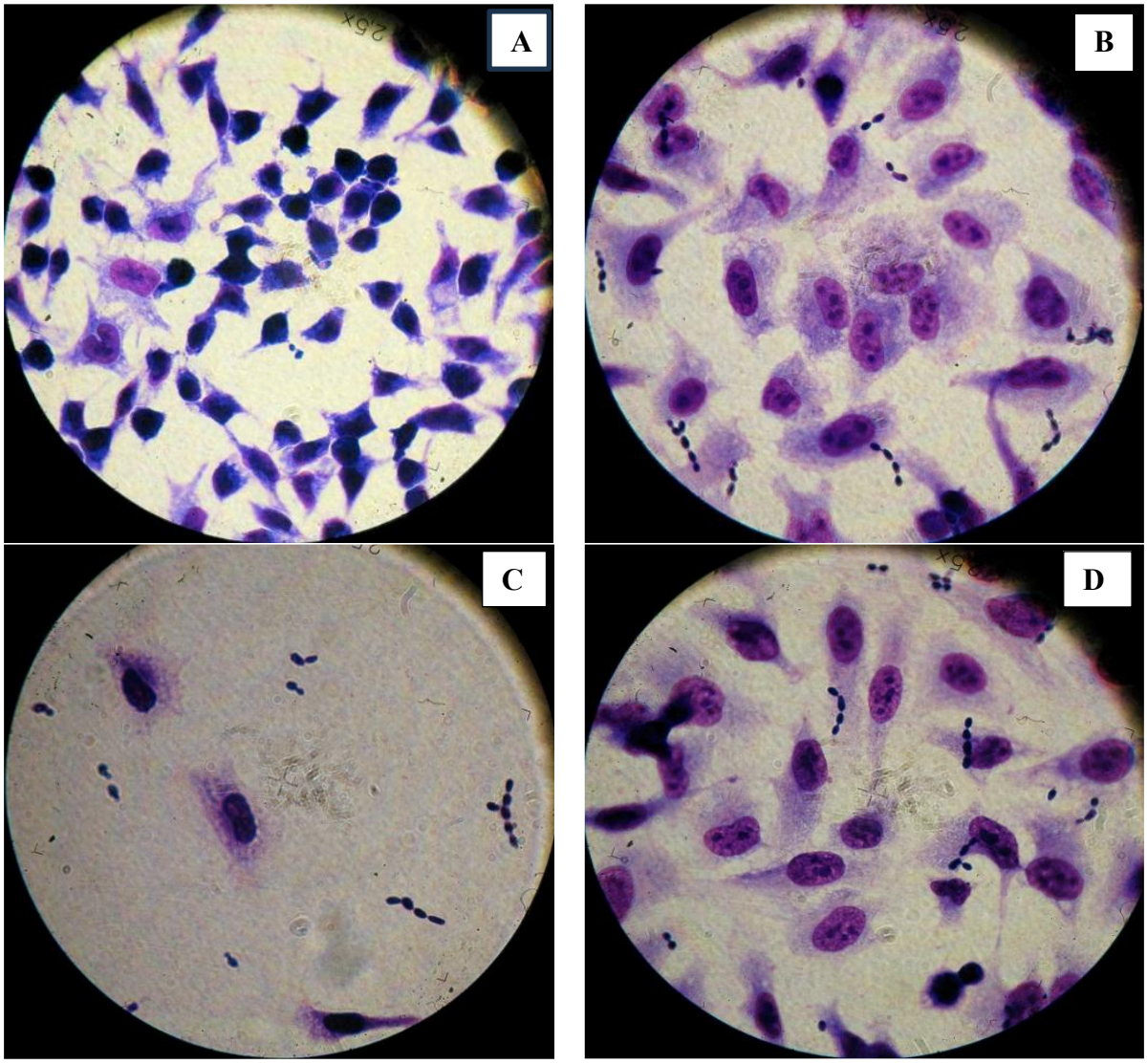

Figure 14.
Representative microscopic images showing the anti-adhesion effect of probiotic supernatants on *Candida albicans* strains: (A) control; (B–C) treated samples; (D) clinical isolate

Conclusions

This study demonstrated the potential for developing functional fermented media based on standard culture media (MRS and EM) supplemented with 0.5% (w/v) lyophilized *Aloe vera*, using probiotic strains *L. rhamnosus* GG, *L. plantarum* and *L. casei*. The presence of Aloe vera positively influenced bacterial growth and supported the maintenance of cell viability throughout fermentation and storage. Of the strains studied, *L. plantarum* and *L. casei* were better adapted and were chosen for further research. Both strains adhered efficiently to HeLa-2 cells and reduced the attachment of *S. aureus* and *C. albicans*. The best performance was achieved with combined inoculum (1:1) especially in EM with *Aloe vera* extract. Besides, storage studies demonstrated an increase in the viability of *L. casei*, indicating the protective action of *Aloe vera*. The probiotic bacteria with *Aloe vera* together may constitute a suitable base for the development of functional fermented products with potential health benefits.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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