

Evaluation of the probiotic properties of lactic acid bacteria isolated from fermented plant-based beverages

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Abstract

Abstract

The aim of this study was to isolate lactic acid bacteria from plant-based fermented beverages and evaluate their probiotic properties. Beet kvass, oat kvass, and monastic kvass were used as the objects of the study. As a result of the research, 36 lactic acid bacterial isolates were obtained, including 13 from beet kvass, 15 from oat kvass, and 8 from monastic kvass. Identification results revealed that strain BK belonged to *Lactiplantibacillus plantarum* / *Lpb. paraplantarum*, whereas strains OK and MK were identified as *Limosilactobacillus fermentum*. Strain BK exhibited the highest tolerance to pH 2.0, maintaining approximately (3.1 ± 0.05) log₁₀ CFU/mL of viable cells after 3 h of incubation. All strains remained viable in the presence of 0.5% bile salts and demonstrated tolerance to 1–10% ethanol. The strains exhibited antagonistic activity against *B. subtilis*, *S. aureus*, and *P. aeruginosa*. The highest antagonistic activity against *S. aureus* was observed for strain BK, with an inhibition zone diameter of 10.23 ± 0.25 mm. At the same time, no proteolytic, amylolytic, or BSH activity was detected in the studied strains. High autoaggregation capacities were observed for all strains, particularly for strain MK. The obtained results indicate the potential of the isolated lactic acid bacterial strains for application in the production of functional foods.

Introduction

Lactic acid bacteria (LAB) play an important role in the food industry and related fields due to their metabolic properties and ability to ensure the microbiological safety and stability of finished products (Lavermicocca et al. 2021; Murata et al. 2025). Representatives of this group of microorganisms are widely used in the production of fermented dairy (Widyastuti et al. 2014), meat (Carneiro et al. 2024; Danylenko et al. 2014), vegetable (Presutto et al. 2026), and cereal products (Petrova and Petrov 2020), as well as beverages (Al-Kharousi 2025; Khablenko et al. 2025), where they participate in the formation of organoleptic characteristics, accumulation of organic acids, synthesis of biologically active compounds, and inhibition of undesirable microflora development.

In addition to their technological significance, LAB are of considerable interest as potential probiotic strains (Khushboo et al. 2023; Sadiq 2022). Current studies indicate the ability of these strains to exert positive effects on the human body by maintaining the balance of the intestinal microbiota, exhibiting antagonistic activity against opportunistic microorganisms, and synthesizing compounds with functional properties (Sanders et al. 2018; Sarita et al. 2025). In this regard, the search for new LAB strains remains a relevant direction of modern research.

According to studies published in recent years, there is a growing interest in the investigation of autochthonous LAB strains isolated from traditional fermented foods and beverages (Munch-Andersen et al. 2026; Xu et al. 2018). These strains are well adapted to specific food substrates, making them promising candidates for application in the production of functional foods (Behera et al. 2018; Gnitsevych and Doronin 2024; Leroy and De Vuyst 2004; Sionek et al. 2023). In addition to facilitating the

fermentation process, autochthonous LAB can enhance the nutritional value and sensory characteristics of food products and confer probiotic properties to the final products (Gnitsevych and Doronin 2024; Leroy and De Vuyst 2004).

Despite the considerable number of studies devoted to the probiotic properties of LAB, most of them focus on strains isolated from dairy raw materials (Taye et al. 2021) or from the gastrointestinal tract of humans and animals (Martín et al. 2003; Sokolova et al. 2025). At the same time, fermented beverages remain a less explored source of LAB, although they are characterized by microbiological diversity and may contain strains with probiotic properties (Khablenko et al. 2025).

The number of studies devoted to the isolation and evaluation of LAB from traditional kvass beverages produced from various types of plant raw materials remains particularly limited (Dluskaya et al. 2008). Kvass beverages are traditional fermented drinks characteristic of Ukraine and other Eastern European countries and have a long history of consumption (Dluskaya et al. 2008; Sagaydak et al. 2016). Due to the specific features of their formulation and spontaneous or mixed fermentation, they may serve as a source of adapted autochthonous LAB with valuable functional properties. However, the microbiological composition of such beverages, as well as the probiotic potential of their LAB isolates, remains insufficiently studied.

Therefore, the aim of this study was to isolate LAB from fermented plant-based beverages and evaluate their probiotic properties in order to determine their potential for application in food biotechnology and in the development of functional food products.

Materials and methods

Sources of LAB

Beet kvass was produced using young beetroot (*Beta vulgaris*). The chopped beetroot was placed in a container and mixed with 2% (w/v) sugar. Boiled water was then added in an amount sufficient to completely cover the beetroot. The container was covered with gauze and left to ferment for 14 days at (25 ± 2) °C.

Oat kvass was produced using common oat (*Avena sativa* L.). The oats were first washed with water, placed in a container, and mixed with warm boiled water at a ratio of 1:5 (w/v). The mixture was then left for 4 days at (25 ± 2) °C. Subsequently, approximately 90% of the liquid was decanted, and the upper layer of oats was removed. An equivalent volume of warm boiled water and 2% (w/v) sugar were added, the mixture was stirred, and fermentation was continued for a further 2 days at the same temperature.

Monastic kvass was prepared according to a traditional recipe using the following ingredient ratio: water – 2.0 L (base component, taken as 100% of the volume); kvass malt – 1 cup (~ 100 g), corresponding to 5% of the water volume; rye flour – 1 cup (~ 120 g), corresponding to 6% of the water volume; raisins – 2 tablespoons (~ 30 g), corresponding to 1.5% of the water volume; lemon – 1 piece (~ 100 g),

corresponding to 5% of the water volume; apple – 1 piece (~ 150 g), corresponding to 7.5% of the water volume; honey – 1 tablespoon (~ 20 g), corresponding to 1% of the water volume. Rye flour was brewed with boiling water (1:1 by volume) and cooled to room temperature, after which crushed apple, lemon, and raisins were added. The mixture was combined with kvass malt, honey, and water and then left to ferment at a temperature of (25 ± 2) °C for 5 days.

Isolation of LAB

For LAB isolation, beverage samples (5–10 mL) were aseptically collected, and serial dilutions (10^{-7} – 10^{-8}) were inoculated by the pour plate method onto solid selective MRS medium prepared according to Simpson et al. (2006). Cultivation was carried out at 37°C for 48–72 hours. Subsequently, colonies were isolated into liquid MRS medium and cultivated at 37°C for 24 hours. To obtain pure cultures, microscopy of the grown isolates and subsequent repeated inoculations onto the indicated media were performed.

Identification of isolates

Various generally accepted morphological and physiological-biochemical methods were used for isolate identification. Morphology was determined by microscopy, while the appearance of surface colonies was evaluated visually. The following characteristics were assessed: catalase production; gelatin liquefaction; ammonia production from arginine; nitrate utilization; gas formation from glucose; growth at 15°C and 45°C; the ability to ferment milk at 15°C, 20°C, 30°C, 37°C, and 45°C; growth at pH 3.5, 4.0, 9.0, and 9.5; growth at sodium chloride concentrations of 1.5%, 3.0%, 6.5%, 10.0%, and 12.0%; and maximum titratable acidity according to Melia et al. (2021).

To determine species affiliation, the ability of the strains to ferment different carbohydrates was evaluated. Cultivation was carried out in a medium containing 1% (v/v) carbohydrates at 37°C for 72 hours. The results were assessed based on the color change of the medium indicator (bromocresol purple). Species identification was performed using the ABIS online service according to the Similarity (%) and Probability (%) indices.

Determination of stress resistance of the isolates

To determine the resistance of the cultures to simulated gastric juice conditions, the effect of low pH (2.0) on their survival was investigated according to Fang et al. (2015). The assessment was carried out by sampling and inoculating serial dilutions onto solid MRS medium at the following time intervals: at the moment of culture inoculation (0 h), and after 1, 1.5, and 3 h of incubation.

Bile resistance was evaluated using a 0.5% bile salt solution according to Farhangfar et al. (2021). Survival was assessed similarly: at the moment of inoculation (0 h), and after 3 and 5 h.

Additionally, ethanol resistance was evaluated by cultivating the cultures in MRS medium supplemented with ethanol at concentrations of 1%, 5%, and 10% (v/v). Medium without ethanol addition (0%) served as the control. Inoculation was performed at 10% (v/v), and cultivation was carried out at 37°C for 24 h. Growth intensity was determined spectrophotometrically at a wavelength of 540 nm.

Enzymatic activity

Proteolytic activity of the isolates was determined using the standard method described by Lim et al. (2019). Amylolytic activity was assessed according to the procedure reported by Kagambèga et al. (2022). Bile salt hydrolase (BSH) activity was determined following the method described by Daliri et al. (2022), with bile salts replaced by purified dry bile at a concentration of 0,5%.

Antagonistic activity

Antagonistic activity was evaluated using a standard method according to Hossain (2024). The following test cultures were used: *B. subtilis* UCM B-901, *E. coli* UCM B-906, *P. aeruginosa* UCM B-900, *S. aureus* UCM B-918 та *C. albicans* UCM Y-1918, *C. utilis* UCM Y-984.

Antibiotic susceptibility

To characterize the complete antibiotic resistance profile, antimicrobial substances from different groups were used, with concentrations indicated in parentheses: benzylpenicillin (10 µg), ampicillin (10 µg), amoxiclav (30 µg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), erythromycin (15 µg), azithromycin (15 µg), tetracycline (30 µg), vancomycin (30 µg), bacitracin (10 µg), polymyxin B (300 U), rifampicin (5 µg), clindamycin (10 µg), ceftriaxone (30 µg), ofloxacin (5 µg), and chloramphenicol (30 µg). The Kirby-Bauer disk diffusion method was used according to Segawa et al. (2020).

Autoaggregation properties

Autoaggregation was determined according to Collado et al. (2008). Optical density was measured after 4, 8, and 24 h of suspension incubation, and the percentage of autoaggregation was calculated using the following formula:

$$A = \left(1 - \frac{A_0 - A_t}{A_0} \right) \cdot 100$$

,

where A_0 is the initial optical density and A_t is the optical density after a certain period of time.

Statistical analysis

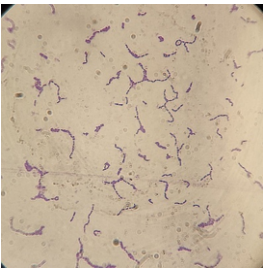
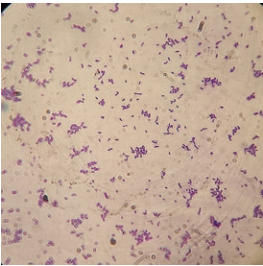
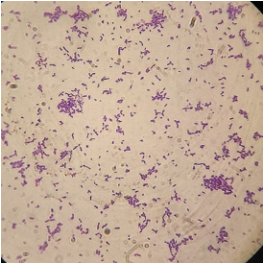
The experiments were performed in three independent replicates. Data are presented as mean ± standard deviation. Statistical significance of differences between mean values was evaluated using Duncan’s test at a significance level of $p < 0.05$. Mean values marked with the same letters did not differ significantly.

Results

Isolation and identification of lactic acid bacteria

At the first stage of the study, lactic acid bacteria were isolated from plant-based fermented beverages. For this purpose, samples were plated onto selective culture media, and individual colonies differing in morphological characteristics were selected. As a result of the study, a number of LAB isolates were obtained, the quantity of which depended on the type of beverage investigated and the characteristics of its microbiota (Table 1).

Table 1
Characteristics of LAB isolates

Source of isolation	Isolate code	Number of isolates	Colony characteristics	Morphological characteristics	Microphotograph
Beet kvass	BK	13	White, round colonies with smooth, shiny surface, regular edges, dense consistency, and pronounced gloss	Short rod-shaped cells arranged in short and long chains	
Oat kvass	OK	15	Light cream or cream-colored, round colonies with smooth surface, regular edges, dense consistency, and slight gloss	Rod-shaped cells of heterogeneous size, ranging from short to long, occurring singly and in short chains	
Monastic kvass	MK	8		Rod-shaped cells, cocco-bacilli, occurring singly	

The isolated cultures exhibited cultural and morphological characteristics typical of LAB. Predominantly white or light cream-colored, round colonies with smooth surfaces and dense consistency were observed. Microscopic examination revealed predominantly rod-shaped cells occurring singly or arranged in short chains. The main cultural and morphological characteristics of the isolates are presented in Table 1.

As a result of the microbiological analysis of the fermented beverages, 36 LAB isolates were obtained, including 13 from the beet kvass, 15 from the oat kvass, and 8 from the monastic kvass. The isolated cultures exhibited cultural and morphological characteristics typical of LAB. Predominantly white or light cream-colored colonies of a round shape with a smooth surface and dense consistency were observed on the culture medium. Microscopic examination revealed that rod-shaped cell forms predominated among the isolated cultures, occurring singly or arranged in short chains. The obtained cultural and morphological characteristics are typical of LAB representatives, particularly bacteria of the *Lactobacillaceae* family, indicating the possibility of identification and investigation of the probiotic properties of the isolated strains.

For further characterization, the physiological and biochemical properties of the isolated cultures were determined. The ability of the isolates to grow at different temperatures, pH values, and NaCl concentrations was evaluated. Catalase activity, milk coagulation, CO₂ production from glucose, ammonia production from arginine, and fermentation of individual carbohydrates were also determined (Table 2).

Table 2
Physiological and biochemical characteristics of the studied cultures

Characteristic		Culture		
		BK	OK	MK
Growth at temperature, °C	15	+	-	-
	45	-	+	+
Catalase		-	-	-
Maximum acidity, °T		188	189	-
Growth on MRS with NaCl, %	1,5	+	+	+
	3,0	+	+	+
	6,5	+ ^w	+	+
	10,0	-	-	+ ^w
	12,0	-	-	-
Milk coagulation ability at temperature, °C	15	-	-	-
	25	+	+	-
	30	+	+	-
	37	+	+	-
	45	-	+	-
Growth in MRS at pH	3,5	+	+	+
	4,0	+	+	+
	9,0	+ ^w	-	+ ^w
	9,5	-	-	-
Gelatin liquefaction		-	-	-
CO ₂ production from glucose		-	+	+
Ammonia production from arginine		-	+	+
Nitrate reduction to nitrite		-	-	-
Carbohydrate fermentation	D-arabinose	-	-	-
	Glucose	+	+	+
	Galatose	+	+	+

Characteristic		Culture		
		BK	OK	MK
	Lactose	+	+	+
	Sucrose	+	+	+
	Fructose	+	+	+
	Maltose	+	+	+
	Mannitol	+	-	-
	Glycerol	-	-	-
	Sorbitol	+	-	-
	Inositol	-	-	-
	Raffinose	-	±	-
	Rhamnose	-	-	-
	D-xylose	-	-	-
	Ribose	+	+	+
	Mannose	+	+	+
	Trehalose	+	-	-
	Melezitose	+	-	-
	Melibiose	+	+	+
	Cellobiose	+	-	-
	Gluconate	+ ^w	+	+

The studied cultures demonstrated differences in their physiological and biochemical characteristics. All three cultures were catalase-negative and were able to grow at pH 3.5 and 4.0. The cultures also differed in their tolerance to elevated NaCl concentrations and in their ability to coagulate milk at different temperatures. The OK and MK cultures produced CO₂ from glucose and ammonia from arginine, whereas the BK culture was negative for both reactions. The carbohydrate fermentation profiles of the cultures also differed (Table 2).

Based on the obtained morphological, cultural, and physiological-biochemical characteristics, and using the ABIS online software, the degree of similarity of the biochemical profile of the studied cultures to the reference species in the database was determined, as well as the probability of their species affiliation, as presented in Table 3.

Table 3
Identification results of LAB cultures using ABIS online software

Culture	Species affiliation	Similarity, %	Probability, %
BK	<i>Lactiplantibacillus plantarum</i> / <i>Lpb. paraplantarum</i>	93,8	99,4
OK	<i>Limosilactobacillus fermentum</i>	94,8	100
MK	<i>Limosilactobacillus fermentum</i>	91,2	99,9

Legend. Similarity – percentage of similarity of the biochemical profile of the studied culture to the reference species profile stored in the ABIS online database; Probability – likelihood of assignment of the studied culture to the corresponding species.

Based on the analysis of a complex of morphological, cultural, and physiological-biochemical characteristics using the ABIS online software, the probable species affiliation of the studied LAB cultures was determined. The BK culture exhibited a high level of similarity of its biochemical profile to representatives of the species *Lactiplantibacillus plantarum* / *Lpb. paraplantarum*, with a Similarity value of 93.8% and a Probability value of 99.4%. The OK and MK cultures showed the highest similarity to the species *Limosilactobacillus fermentum*, with Probability values of 100% and 99.9%, respectively.

The obtained results are consistent with the data of the physiological and biochemical studies, particularly the ability of the OK and MK cultures to produce CO₂ from glucose, synthesize ammonia from arginine, and grow at 45°C, which are characteristic features of obligately heterofermentative representatives of the genus *Limosilactobacillus*. In contrast, the BK culture was characterized by the absence of CO₂ production and the inability to grow at 45°C, which is consistent with the typical properties of representatives of the *Lactiplantibacillus plantarum* group.

Study of stress resistance of lactic acid bacteria cultures

An important characteristic of potential probiotic cultures is their ability to maintain viability under the influence of stress factors typical of the human gastrointestinal tract and fermented food systems. The main factors include the low pH values of gastric juice, the presence of bile salts in the intestine, as well as the effect of ethanol, which may be characteristic of fermented beverages.

In this regard, the resistance of the studied LAB cultures to pH 2.0 was evaluated, and the results are presented in Fig. 1.

It was established that all studied cultures were characterized by a decrease in the number of viable cells during incubation at pH 2.0. The BK culture (*Lactiplantibacillus plantarum* / *Lpb. paraplantarum*) demonstrated the highest resistance to the acidic environment, with the number of viable cells after 3 h of incubation remaining at approximately $3.1 \pm 0.05 \log_{10}$ CFU/mL. At the same time, the OK and MK cultures (*Limosilactobacillus fermentum*) were characterized by lower survival rates. In particular, for the MK culture, the number of viable cells after 3 h of incubation decreased to approximately $0.98 \pm 0.07 \log$ CFU/mL.

The next series of experiments was devoted to the investigation of the resistance of LAB cultures to 0.5% bile salts. The incubation time was 5 h. The results of the study are presented in Fig. 2.

It was established that after incubation in a medium containing 0.5% bile salts, a gradual decrease in the number of viable cells was observed for all studied cultures (Fig. 2). After 3 and 5 h of incubation, the BK culture (*Lactiplantibacillus plantarum* / *Lpb. paraplantarum*) demonstrated the highest survival rates, whereas the OK and MK cultures (*Limosilactobacillus fermentum*) were characterized by lower, but similar, values.

To evaluate the ethanol resistance of the studied LAB cultures, the intensity of their growth in media containing different ethanol concentrations was determined. The obtained results are presented in Fig. 3.

The studied LAB cultures responded differently to the effect of ethanol. It was established that an ethanol concentration of 1% had virtually no effect on the growth intensity of the BK, OK, and MK cultures. When the ethanol concentration was increased to 5%, a gradual decrease in growth intensity was observed, most pronounced for the MK culture (*Limosilactobacillus fermentum*).

A further increase in ethanol concentration to 10% resulted in significant growth inhibition of all studied cultures. Under these conditions, the BK culture (*Lactiplantibacillus plantarum* / *Lpb. paraplantarum*) demonstrated the highest optical density values, indicating its greater resistance to ethanol. At the same time, the OK and MK cultures (*Limosilactobacillus fermentum*) were characterized by lower growth intensity, especially the MK culture.

Study of the enzymatic activity of lactic acid bacteria

The obtained data on the enzymatic activities of the studied strains make it possible to assess their potential applications in food production (proteolytic and amylolytic activities) as well as their probiotic potential (BSH activity). The results of the enzymatic activity assays are presented in Table 4.

Table 4
Enzymatic activity of LAB cultures

Enzymatic activity	Tested culture		
	BK	OK	MK
Proteolytic	-	-	-
Amylolytic	-	-	-
BSH	-	-	-

None of the studied cultures exhibited proteolytic, amylolytic, or BSH activity (Table 4).

Antagonistic activity of LAB cultures

To evaluate the antagonistic properties of the studied LAB cultures against opportunistic microorganisms, corresponding experiments were carried out. *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus*, as well as the yeasts *Candida albicans* and *C. utilis*, were used as test cultures. The results are presented in Table 5.

Table 5
Antagonistic activity of LAB cultures against opportunistic microorganisms

Opportunistic microorganism	Tested culture		
	BK	OK	MK
<i>B. subtilis</i> UCM B-901	8,30 ± 0,36 ^a	5,23 ± 0,25 ^b	5,33 ± 0,31 ^b
<i>E. coli</i> UCM B-906	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a
<i>P. aeruginosa</i> UCM B-900	10,07 ± 0,31 ^a	0,00 ± 0,00 ^c	9,23 ± 0,25 ^b
<i>St. aureus</i> UCM B-918	10,23 ± 0,25 ^a	6,57 ± 0,21 ^b	5,30 ± 0,36 ^c
<i>C. albicans</i> UCM Y-1918	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a
<i>C. utilis</i> UCM Y-984	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a	0,00 ± 0,00 ^a

It was established that all studied cultures exhibited antagonistic activity against *B. subtilis* and *S. aureus*. The highest antagonistic activity against *S. aureus* was demonstrated by the BK culture (*Lactiplantibacillus plantarum* / *Lpb. paraplantarum*), for which the diameter of the inhibition zone was (10.23 ± 0.25) mm.

At the same time, the BK and MK cultures (*Limosilactobacillus fermentum*) exhibited pronounced antagonistic activity against *P. aeruginosa*, whereas no antagonistic activity against this test culture was detected for the OK culture. Against *B. subtilis*, the BK culture was also characterized by higher antagonistic activity compared to the OK and MK cultures. The studied cultures did not exhibit antagonistic activity against *E. coli*, *C. albicans*, or *C. utilis*, for which no growth inhibition zones were observed.

Study of the antibiotic susceptibility of lactic acid bacteria

To evaluate the safety and potential applicability of LAB cultures in the food industry, their susceptibility to antibiotics of different groups was investigated. The results are presented in Table 6.

Table 6
Antibiotic susceptibility of LAB cultures

Antibiotic	Tested culture		
	BK	OK	MK
PEN	R	R	R
AMP	S	S	S
AMC	S	S	S
STR	R	R	R
GEN	R	R	R
KAN	R	R	R
CMP	S	S	S
ERY	S	S	S
AZY	nd	nd	Nd
TET	S	S	S
VAN	R	R	R
BAC	R	R	R
POL	R	R	R
RIF	S	S	S
CLI	nd	nd	nd
CEF	S	S	S
OFL	MS	R	R

Legend. R – resistant; MS – moderately susceptible; S – susceptible; nd – not defined by the standard; PEN – penicillin, AMP – ampicillin, AMC – amoxicillin/clavulanic acid, STR – streptomycin, GEN – gentamicin, KAN – kanamycin, CMP – chloramphenicol, ERY – erythromycin, AZY – azithromycin, TET – tetracycline, VAN – vancomycin, BAC – bacitracin, POL – polymyxin B, RIF – rifampicin, CLI – clindamycin, CEF – cephalosporin, OFL – ofloxacin.

It was established that all studied cultures were resistant to penicillin, aminoglycosides (streptomycin, gentamicin, and kanamycin), vancomycin, bacitracin, and polymyxin B. Such results are typical of representatives of the *Lactobacillaceae* family and may be associated with the structural features of the cell wall and intrinsic resistance to certain groups of antibiotics.

At the same time, the strains were susceptible to most other antibiotics. In particular, the BK, OK, and MK cultures were susceptible to chloramphenicol, erythromycin, tetracycline, and rifampicin. Differences between the cultures were observed for ofloxacin: the BK culture was characterized by moderate susceptibility, whereas the OK and MK cultures were resistant.

Autoaggregation ability of LAB cultures

The final stage in the evaluation of the functional properties of the studied LAB cultures was the assessment of their autoaggregation ability. The obtained results regarding the autoaggregation capacity of the investigated LAB cultures provide an indication of their potential for adhesion and biofilm formation, which are important characteristics of probiotic microorganisms. The results of the autoaggregation assay are presented in Fig. 4.

It was established that the autoaggregation values of all studied cultures gradually increased with increasing incubation time. After 4 h, the highest autoaggregation values were observed for the MK culture (*Limosilactobacillus fermentum*), whereas the BK (*Lactiplantibacillus plantarum* / *Lpb. paraplantarum*) and OK (*Limosilactobacillus fermentum*) cultures were characterized by lower values. After 24 h of incubation, the MK culture also demonstrated the highest level of autoaggregation, indicating a high ability of this culture for intercellular interaction and aggregate formation. The BK and OK cultures were characterized by slightly lower, but still high, autoaggregation values.

Discussion

Diversity and identification of LAB isolates

The isolation of 36 LAB cultures from the three fermented plant-based beverages demonstrates the presence of diverse LAB populations associated with these substrates. The predominance of rod-shaped cells and the observed cultural characteristics are consistent with the typical morphology of members of the *Lactobacillaceae* family.

The physiological and biochemical profiles provided additional evidence supporting the identification of the studied cultures. The ability of the OK and MK cultures to produce CO₂ from glucose and ammonia from arginine, together with their growth at 45°C, is consistent with characteristics associated with obligately heterofermentative LAB and supports their assignment to *Limosilactobacillus fermentum*. In contrast, the absence of CO₂ production and inability to grow at 45°C in the BK culture are consistent with characteristics of the *Lactiplantibacillus plantarum* group.

The identification results are consistent with literature data on the microbiota of plant-based fermented beverages. Members of the *Lactiplantibacillus plantarum* group are frequently reported among the microorganisms associated with fermented vegetable products and beverages, including fermented juices and kvass (Garmasheva et al. 2015; Livinska et al. 2016; Xing et al. 2025). For example, *Lpb. plantarum* and *Pc. pentosaceus* were isolated from traditional fermented watermelon juice (Y.-S. Chen et al. 2016).

The identification of the OK and MK cultures as *Limosilactobacillus fermentum* is also consistent with observations for fermented cereal beverages. *Lmb. fermentum* is frequently associated with the fermentation of cereal substrates and products such as boza (Melda et al. 2021; Rasheed et al. 2021; Zhao et al. 2025). Other LAB, including *Lpb. plantarum*, *Lpb. pentosus*, and *Lvb. brevis*, have also been reported in similar fermented products (Rasheed et al. 2021; Zhao et al. 2025).

Stress tolerance and probiotic potential

The differences in acid resistance observed among the studied cultures indicate a strain-dependent response to low pH. The higher survival of BK compared with OK and MK may be associated with differences in the physiological characteristics of the corresponding LAB groups. Representatives of the *Lactiplantibacillus plantarum* group are frequently characterized by relatively high tolerance to acidic conditions, whereas *Limosilactobacillus fermentum* strains may demonstrate greater sensitivity to prolonged exposure to low pH. These observations are consistent with previous studies reporting substantial variability in acid resistance among *Lpb. plantarum* strains at pH 2.0 and reduced viability of *Lmb. fermentum* under prolonged acidic exposure (Beganović et al. 2014; Vitali et al. 2012; Panicker et al. 2018).

All three cultures retained viability in the presence of 0.5% bile salts, although the BK culture demonstrated the highest survival. This finding indicates that the studied cultures possess different degrees of tolerance to bile salts, which may be relevant when considering their potential probiotic properties. The results are consistent with previous reports describing high bile tolerance among representatives of *Lpb. plantarum* and certain strains of *Lmb. fermentum* (Coelho-Rocha et al. 2023; Kim et al. 2022; Pato et al. 2022; Sharafi et al. 2015).

The ethanol-resistance results further demonstrated the strain-specific nature of the response of LAB to environmental stress. The relatively higher tolerance of BK to 5–10% ethanol compared with OK and MK may be relevant to its potential use in fermented beverages. The observed differences are generally

consistent with literature data describing the ability of certain *Lpb. plantarum* and *Lmb. fermentum* strains to maintain growth under elevated ethanol concentrations (X. Chen et al. 2020; Kang et al. 2024; Li et al. 2025).

Overall, the stress-resistance results demonstrate that the three cultures differ in their tolerance to acidic conditions, bile salts, and ethanol. Among the studied cultures, BK showed the most pronounced tolerance across the tested stress conditions, whereas MK generally demonstrated lower tolerance, particularly under acidic and ethanol stress.

Functional properties of LAB cultures

The absence of amylolytic activity in the studied cultures is consistent with previous observations that this trait is relatively uncommon among LAB (Xu et al. 2020). Similarly, most *Lactiplantibacillus plantarum* strains have been reported to lack proteolytic activity, although weak or moderate activity may occur in some strains (Kuerman et al. 2024). Proteolytic activity has also been reported only infrequently for *Limosilactobacillus fermentum* (Sasaki et al. 1995).

The absence of BSH activity in all three cultures indicates that this characteristic was not detected under the conditions used in the present study. BSH activity is known to be strongly strain-dependent and influenced by the origin of LAB isolates; it has been reported in fewer than half of the members of the *Lactobacillaceae* family (Song et al. 2019).

Antagonistic activity of LAB cultures

The antagonistic activity observed in the present study demonstrates a strain-dependent inhibitory effect of the investigated LAB cultures against the tested microorganisms. The particularly pronounced activity of BK against *S. aureus* and *B. subtilis*, together with the activity of BK and MK against *P. aeruginosa*, indicates that the inhibitory spectrum differed among the studied cultures.

The absence of inhibition of *E. coli*, *C. albicans*, and *C. utilis* further demonstrates the specificity of the antagonistic activity. Such differences may reflect variation in the antimicrobial compounds produced by individual LAB strains and differences in the susceptibility of the target microorganisms.

The obtained results are generally consistent with previous reports describing antagonistic activity of *Lactiplantibacillus plantarum* and certain *Limosilactobacillus fermentum* strains against Gram-positive and some Gram-negative opportunistic microorganisms (Beganović et al. 2014; Hu et al. 2019; Sharafi et al. 2015).

Antibiotic susceptibility of LAB cultures

The antibiotic susceptibility profiles demonstrated that the studied cultures had similar responses to several antimicrobial agents, but differences were observed for ofloxacin. Such strain-dependent variation in antibiotic susceptibility is consistent with the previously reported variability among LAB.

The observed resistance to aminoglycosides and vancomycin, together with susceptibility to tetracycline, erythromycin, and chloramphenicol, is generally consistent with published data for representatives of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* (Beganović et al. 2014; Carboni et al. 2025; dos Santos et al. 2021).

The antibiotic susceptibility profile should be considered when evaluating the potential application of LAB cultures in food production, particularly because the presence of antibiotic resistance may affect their safety assessment.

Autoaggregation ability and probiotic potential

The high autoaggregation ability observed for all three cultures indicates differences in their capacity for cell–cell aggregation and may be relevant to their potential adhesive properties. The particularly high autoaggregation of MK suggests that this characteristic may be strain-dependent even among cultures identified as *Limosilactobacillus fermentum*.

The obtained results are consistent with previous reports showing that representatives of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* can exhibit high autoaggregation ability, with substantial variation between individual strains (Collado et al. 2008; Holubchik et al. 2025; Liu et al. 2022).

Overall, the autoaggregation results complement the stress-resistance and antagonistic-activity data and provide additional evidence for the functional potential of the studied LAB cultures.

Conclusions

The study resulted in the isolation and characterization of lactic acid bacteria from plant-based fermented beverages. Based on their morphological, physiological, and biochemical characteristics, the isolates were assigned to the *Lactiplantibacillus plantarum* / *Lpb. paraplantarum* group and *Limosilactobacillus fermentum*. The studied cultures demonstrated different levels of tolerance to acidic conditions, bile salts, and ethanol, with the BK culture showing the highest overall stress tolerance. All cultures exhibited antagonistic activity against selected microorganisms, particularly *B. subtilis* and *S. aureus*, while BK and MK also showed activity against *P. aeruginosa*. No proteolytic, amylolytic, or BSH activity was detected. The studied cultures also demonstrated high autoaggregation ability and distinct antibiotic susceptibility profiles. Overall, the results indicate that the investigated LAB cultures possess promising functional characteristics and warrant further investigation to assess their suitability for potential application in functional food production.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AK: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft; DH: Investigation, Writing – review & editing; SD: Methodology, Supervision, Writing – review & editing; OD: Methodology, Supervision, Writing – review & editing; OIY: Supervision, Validation, Writing – review & editing.

All authors read and approved the final manuscript.

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Figures

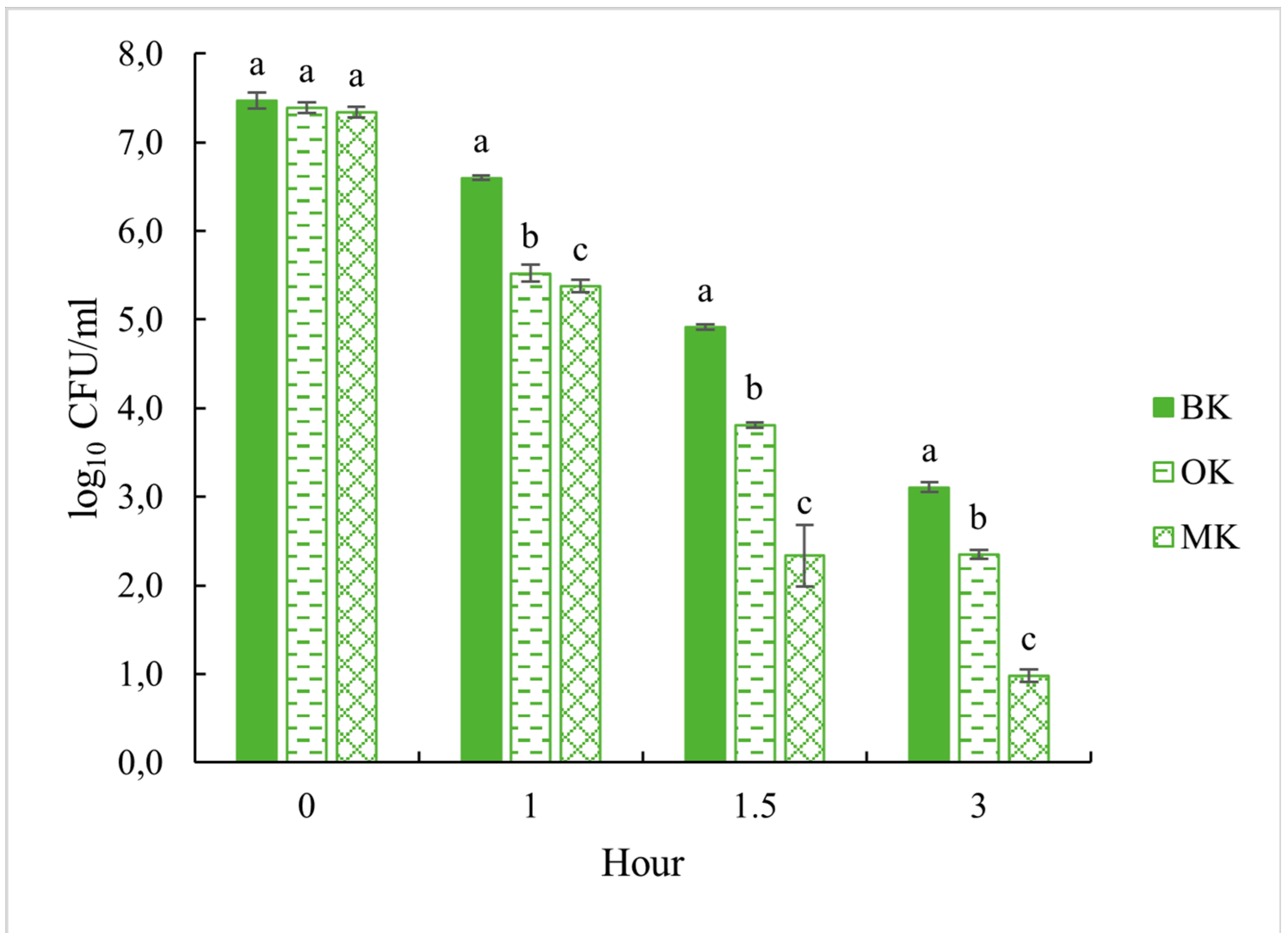


Figure 1

Survival of LAB cultures at pH 2.0

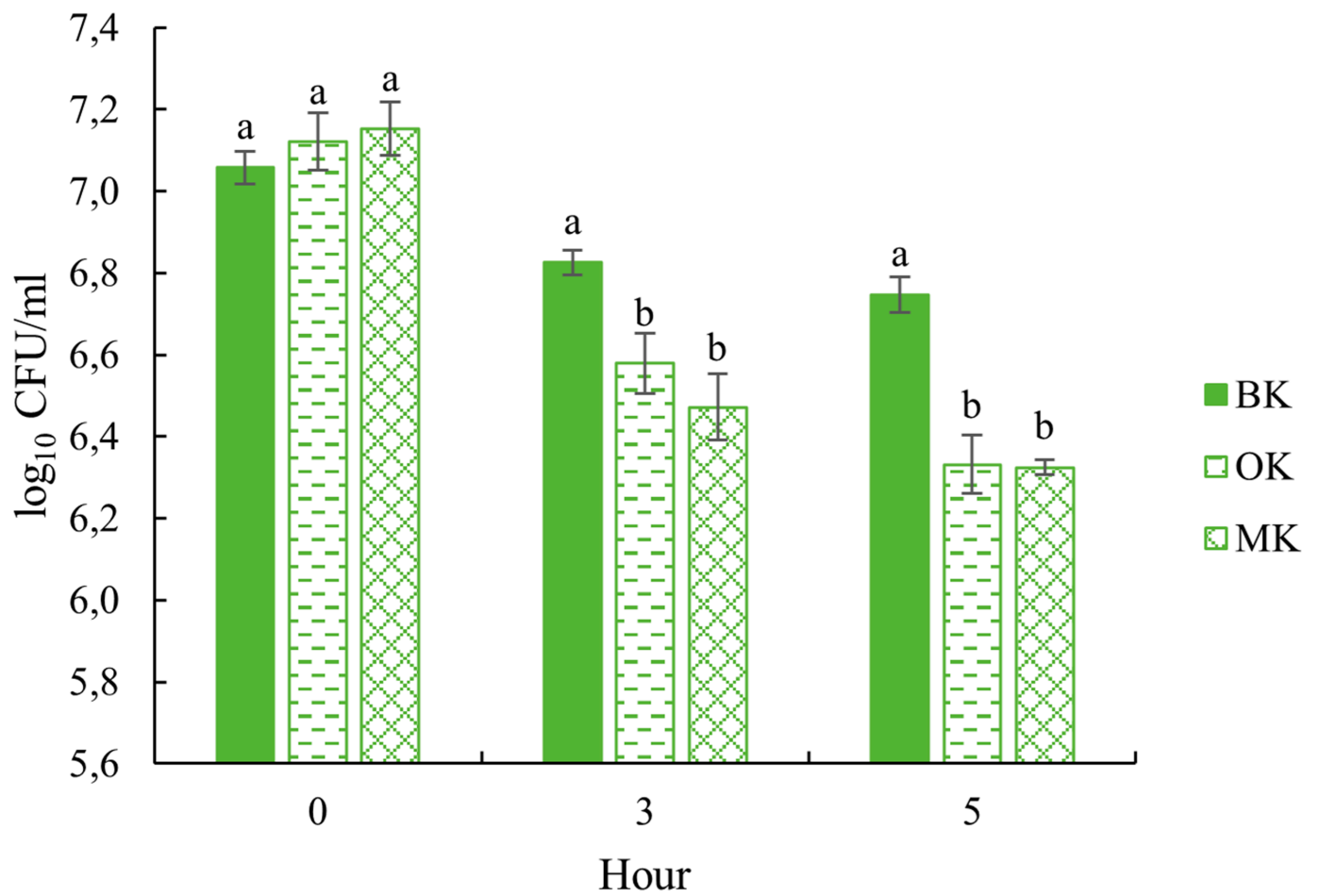


Figure 2

Survival of LAB cultures in medium containing 0.5% bile salts

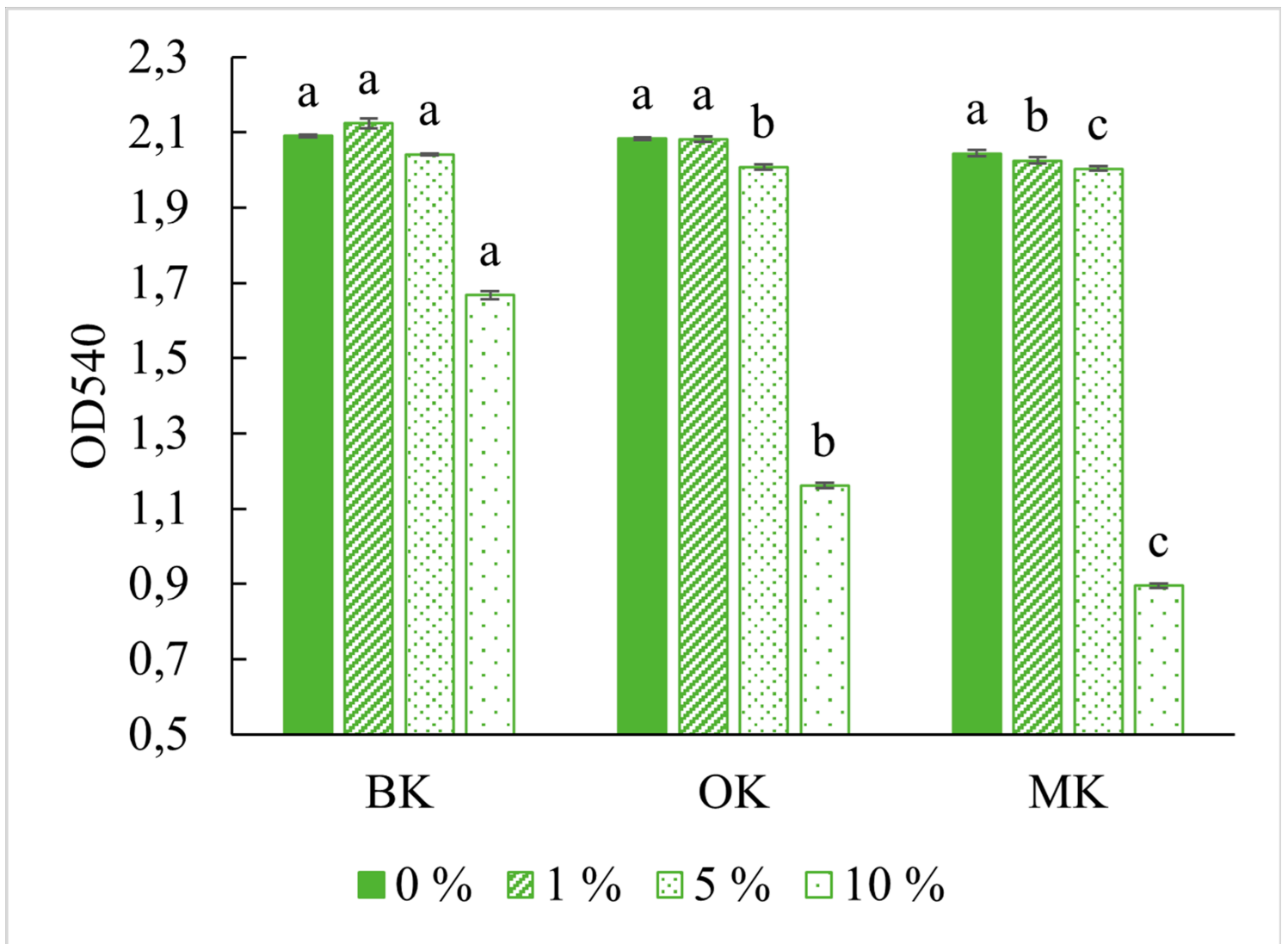


Figure 3

Effect of different ethanol concentrations on the growth of LAB cultures

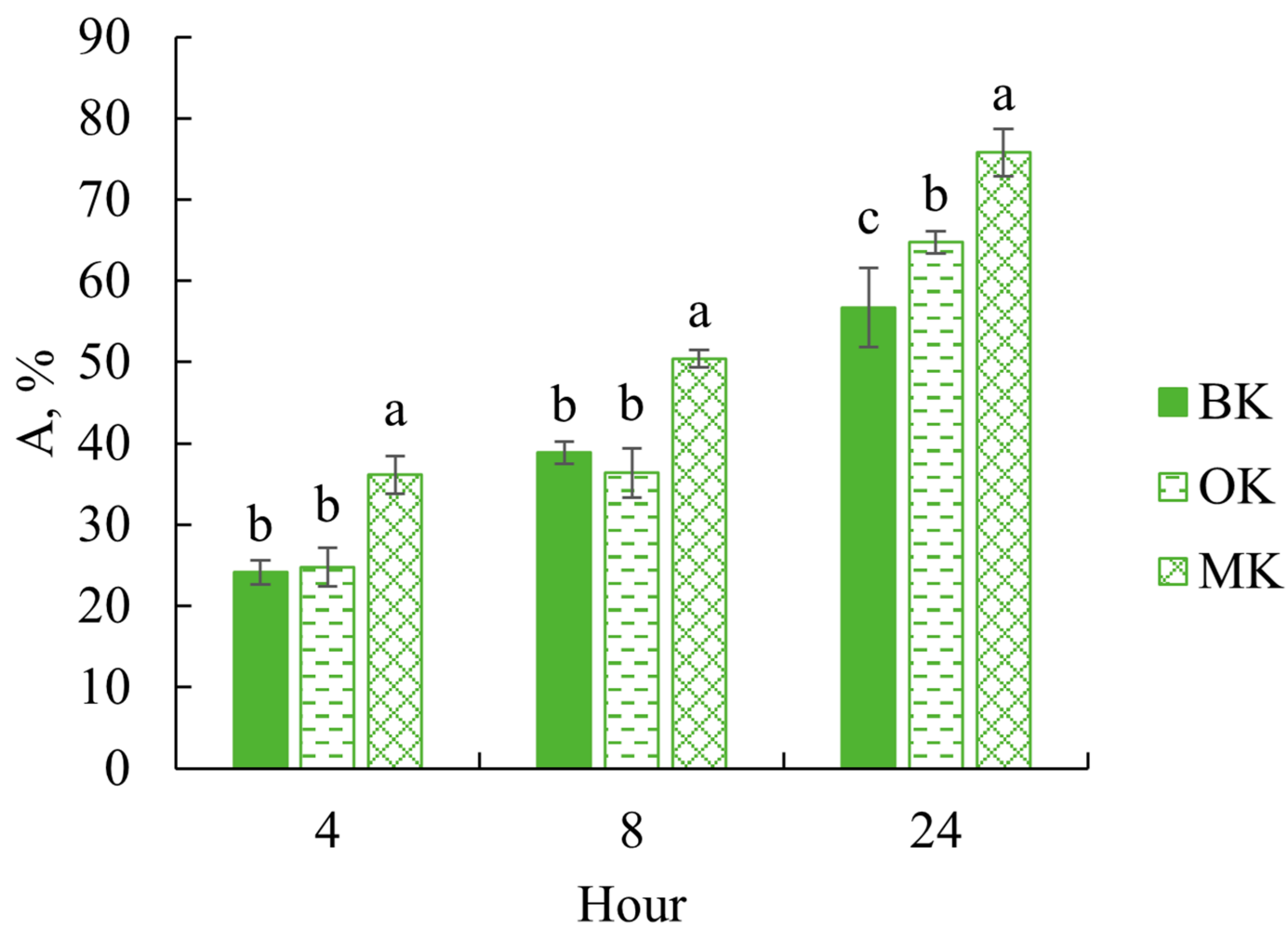


Figure 4

Autoaggregation of the studied lactic acid bacteria cultures