

FUNCTIONAL CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED WATERMELON JUICE

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*Fermented plant-based products are an important source of lactic acid bacteria (LAB); however, most studies focus on traditional substrates, while spontaneously fermented fruit juices, particularly watermelon juice, remain poorly investigated. The limited data on the species composition and probiotic properties of lactic acid bacteria isolated from such substrates determine the need for further research. To isolate, identify, and evaluate the probiotic properties of a LAB isolate obtained from fermented watermelon juice. Standard microbiological and physiological-biochemical research methods were used. Isolation and cultivation were performed on MRS medium at 37 °C. Identification was carried out based on morphological and physiological-biochemical characteristics using Bergey's Manual and ABIS online software. Stress resistance was assessed by cell survival at pH 2.0 and in the presence of 0.5 % bile salts. Antagonistic activity was determined by the agar diffusion method, antibiotic susceptibility by the disk diffusion method, and autoaggregation properties by changes in the optical density of the cell suspension. A lactic acid bacteria isolate was obtained from fermented watermelon juice and identified as *Limosilactobacillus fermentum* based on a combination of morphological and physiological-biochemical characteristics. Low resistance to acidic conditions (pH 2.0) and high resistance to bile salts (0.5 %) were established. The isolate exhibited antagonistic activity against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and showed no activity against Gram-negative microorganisms or yeasts of the genus *Candida*. A typical antibiotic susceptibility profile and moderate autoaggregation ability (up to 42.93 %) were determined. The obtained results may be used for further investigation of the probiotic properties of the *Lmb. fermentum* strain, as well as in the development of functional fermented beverages and biotechnological products.*

Keywords: lactic acid bacteria, fermented plant substrates, probiotic properties, fermented watermelon juice, biotechnology.

Introduction. Fermented plant-based products are an important source of lactic acid bacteria (LAB), which are widely used in the food industry and are considered promising probiotic microorganisms. A considerable number of studies have focused on the microbiota of traditional fermented substrates, namely vegetable raw materials (cabbage, cucumbers) (Valentino et al., 2024) and cereal-based beverages (Yehuala et al., 2024), which are typically dominated by representatives of the genera *Lactiplantibacillus* (Lin et al., 2025), *Levilactobacillus* (Ferdiansyah et al., 2025; Zhao et al., 2025), and *Limosilactobacillus* (Ferdiansyah et al., 2025; Khablenko et al., 2025; Zhao et al., 2025). In light of recent taxonomic and genetic studies that have expanded the understanding of LAB distribution (Zheng et al., 2020), plant raw materials have gained increased importance as a promising source for the isolation of

new, functionally valuable LAB strains (D. Holubchyk et al., 2025; D. S. Holubchyk, 2025; Putria et al., 2026).

Although most studies focus on “typical” sources of isolation, such as fermented dairy products (Luz et al., 2021), meat raw materials (Danylenko, 2014), and the microbiota of the human (Ryan et al., 2008) and animal gastrointestinal tract (Zhang et al., 2025), non-traditional plant-based fermented substrates remain insufficiently explored. Food products such as spontaneously fermented fruit juices are significantly less studied in the context of LAB isolation and characterization (Li et al., 2021; Yien Ong et al., 2012). The limited available studies indicate the possibility of isolating individual LAB representatives; however, data on their species composition, physiological and biochemical properties, and probiotic potential remain scarce.

At the same time, it is known that non-traditional sources may harbor LAB strains with unique properties, such as enhanced stress resistance, antagonistic activity, and adhesion potential, which accounts for the growing interest in their study (Prabhurajeshwar & Chandrakanth, 2017). Moreover, autochthonous LAB isolated from specific food products are generally better adapted to the conditions of their respective environments and can be used both in the development of starter cultures for fermented beverages and as potential probiotics (Leroy & De Vuyst, 2004; Sionek et al., 2023).

Thus, the limited data on LAB isolated from spontaneously fermented fruit juices, particularly watermelon juice, as well as their potential probiotic properties, determines the relevance of research in this area.

The purpose of the study was to isolate, identify, and evaluate the probiotic properties of a LAB isolate obtained from fermented watermelon juice.

Materials and methods. Fermented watermelon juice was obtained by crushing the pulp of watermelon (*Citrullus lanatus*). The pulp was pressed to obtain juice, which was then left to ferment for 3 days at a temperature of (30 ± 2) °C. For LAB isolation, beverage samples ($5\text{--}10\text{ cm}^3$) were collected under aseptic conditions.

Standard microbiological methods were used for the isolation of LAB. Pure cultures were obtained by plating serial dilutions of liquid samples onto MRS agar prepared according to (Simpson et al., 2006). Both pour plate and spread plate techniques were applied. Incubation was carried out at 37 °C for 48–72 h. Microscopic preparations were made using conventional methods, and Gram staining was performed. Observations were conducted using a Motic (Fisher Bioblock) microscope at $\times 1000$ magnification. In cases where cell heterogeneity or contamination was detected, re-streaking onto appropriate media was performed.

For the selection and identification of the obtained pure cultures, standard morphological and physiological–biochemical methods were applied. The following characteristics were determined: cell morphology (shape and size), catalase production, gelatin liquefaction, ammonia production from arginine, nitrate utilization, gas formation from glucose, growth at 15 °C and 45 °C according to (Gerhardt, 1994), growth and milk fermentation ability at 15 °C, 20 °C, 25 °C, 30 °C, 37 °C, and 45 °C according to (Gerhardt, 1994; Niguse et al., 2026), growth at different sodium chloride concentrations 1.5 %, 3.0 %, 6.5 %, 10.0 %, and 12.0 % (Menconi et al., 2014; Gerhardt, 1994), and growth at pH 3.5, 4.0, 9.0, and 9.5 (Gerhardt, 1994).

Identifications performed according to “Bergey’s Manual of Determinative Bacteriology” and Holzappel. To determine the percentage similarity with other species and the probability of belonging to a particular species, based on the obtained morphological-cultural and physiological–biochemical characteristics, ABIS online software was used.

To assess LAB stress resistance, the culture was grown in liquid MRS medium for 18 h at 37 °C. The resulting cell biomass was separated by centrifugation at 5000 rpm for 15 min, washed twice with sterile phosphate buffer (pH 7.2), and resuspended in the same buffer.

Acid tolerance was determined by incubating the cells in a solution with pH 2.0 (Yu et al., 2013) at 37 °C for 3 h. Viability was assessed by plating on MRS agar after 0, 1, 1.5, and 3 h of incubation

Bile tolerance was evaluated in a 0.5 % bile solution (Prabhurajeshwar & Chandrakanth, 2019) at 37 °C for 5 h. The number of viable cells was determined by plating on MRS agar after 0, 3, and 5 h of incubation.

The antagonistic activity of the culture was assessed by the agar diffusion method according to (Hossain, 2024) against test cultures of opportunistic bacteria (*B. subtilis* UCM B-901, *E. coli* B-906, *P. aeruginosa* UCM B-900, *S. aureus* UCM B-918) and yeasts of the genus *Candida* (*C. albicans* UCM Y-1918, *C. utilis* Y-984). After incubation at 37 °C for 24 h, the diameters of growth inhibition zones (mm) were measured.

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method (Segawa et al., 2020). The cell suspension was standardized to 0.5 McFarland, evenly spread on MRS agar, and antibiotic discs of different groups were applied. Incubation was carried out at 37 °C for 48 h, after which the diameters of growth inhibition zones were measured. The results were interpreted according to the accepted criteria given in (Charteris et al., 1998).

Autoaggregation properties were evaluated according to (Collado et al., 2008). The cells were washed with phosphate buffer (pH 7.2) and resuspended to an optical density of $OD_{600} = 0.5 \pm 0.05$ ($\approx 10^8$ CFU/cm³). The suspension was incubated at 37 °C for 24 h. OD_{600} values were measured after 4, 8, and 24 h. The percentage of autoaggregation was calculated using the 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 at the corresponding time point.

All experiments were performed in triplicate. The results are presented as mean \pm standard deviation. Statistical significance of differences between means

was determined using one-way analysis of variance with Duncan's test at a significance level of $p < 0.05$. Mean values marked with different letters differ significantly.

Results and discussion. Twelve LAB isolates were obtained from fermented watermelon juice, all of which exhibited similar morphological and cultural characteristics. All isolates were Gram-positive and catalase-negative. Due to the absence of significant differences among the isolates, one representative isolate was selected for further study. The cell morphology of isolate W is shown in Figure 1.

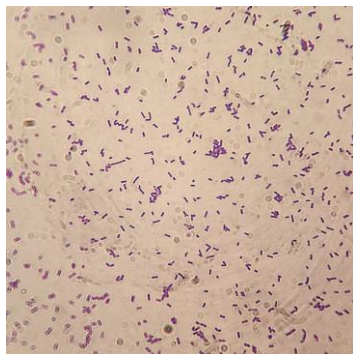


Fig. 1. Cell morphology of isolate W ($\times 1000$)

As shown in Figure 1, the cells of the isolate exhibited a rod-shaped morphology, occurring singly or in short chains. The cells were uniformly stained, varied in size, and no spores were observed. Based on morphological characteristics, the isolate displayed features typical of most representatives of the family *Lactobacillaceae*.

Isolate W demonstrated growth in liquid MRS medium typical for LAB, appearing as uniform turbidity throughout the medium, which indicates a facultatively anaerobic mode of metabolism. In deep agar cultures, the formation of boat-shaped and disc-shaped colonies was observed, with sizes ranging from 1 to 4 mm. On the surface of agarized medium, the isolate formed light cream to cream-colored, round colonies with smooth edges, a smooth surface, dense consistency, and slight gloss.

Identification of isolate W was performed based on physiological and biochemical tests using Bergey's Manual and ABIS online software. The results are presented in Table 1.

It was established that isolate W was unable to grow at 15 °C but grew at 45 °C, indicating thermotolerance. The culture did not liquefy gelatin and did not reduce nitrates, which is typical for representatives of the family *Lactobacillaceae*. The isolate was capable of growth in media with elevated NaCl concentrations (up to 6.5 %), indicating osmotolerance.

The production of CO₂ from glucose and ammonia from arginine is a characteristic feature of obligately heterofermentative LAB (Holzapfel &

Wood, 2014). The isolate fermented glucose, lactose, sucrose, fructose, maltose, and ribose, whereas negative results were obtained for carbohydrates such as mannitol, sorbitol, trehalose, glycerol, cellobiose, and mannose. For certain carbohydrates (galactose), a weakly positive reaction was observed.

Table 1.

Physiological and biochemical characteristics of the studied isolate

Characteristic		Result
Growth at temperatures, °C	15	-
	45	+
Growth on MRS with NaCl, %	1,5	+
	3,0	+
	6,5	+ ^w
	10,0	-
	12,0	-
Milk fermentation ability at temperatures, °C	15	-
	25	-
	30	-
	37	-
Growth in MRS at pH	45	-
	3,5	+
	4,0	+
	9,0	+ ^w
Growth in MRS at pH	9,5	+ ^w
	Gelatin liquefaction	
CO ₂ production from glucose		+
Ammonia production from arginine		+
Nitrate reduction		-
Carbohydrate fermentation	D-arabinose	-
	Glucose	+
	Galactose	+ ^w
	Lactose	+
	Sucrose	+
	Fructose	+
	Maltose	+
	Mannitol	-
	Glycerol	-
	Sorbitol	-
	Inositol	-
	Raffinose	+
	Rhamnose	-
	D-xylose	-
	Ribose	+
	Manose	-
	Trehalose	-
	Melezitose	-
Melibiose	+	
Cellobiose	-	
Gluconate	+	

Based on the overall physiological and biochemical characteristics, isolate W was preliminarily classified as an obligately heterofermentative lactic acid bacterium. According to the analysis using ABIS online software, isolate

W most likely belongs to the species *Limosilactobacillus fermentum*: the similarity of the biochemical profile of the studied culture to the reference profile was 91.6 %, and the probability of belonging to this species was 99.2 %.

The isolation of LAB from fermented watermelon juice remains poorly studied, as most available works focus on other plant substrates, such as fermented vegetables (Chen, 2021) or cereal-based beverages (Khablenko et al., 2025). It is known that fermented plant products are typically dominated by *Lactiplantibacillus plantarum* (Paramithiotis, 2025), whereas data on the microbiota of fermented watermelon are limited. The few available studies report the isolation of individual LAB representatives from this substrate.

For example, study (Y.-S. Chen et al., 2016) indicates the isolation of *Lpb. plantarum* strains from fresh and fermented watermelon. At the same time, a number of studies report that *Lmb. fermentum* is a typical representative of the microbiota of fermented plant-based beverages, particularly cereal-based ones (Farid et al., 2026; Rasheed et al., 2021; Zhao et al., 2025). Thus, the obtained results complement the limited literature data on the species composition of LAB in fermented watermelon juice.

To evaluate the probiotic potential of isolate W, its stress resistance was investigated, as this is one of the key criteria for the selection of probiotic strains. The results are presented in Figure 2.

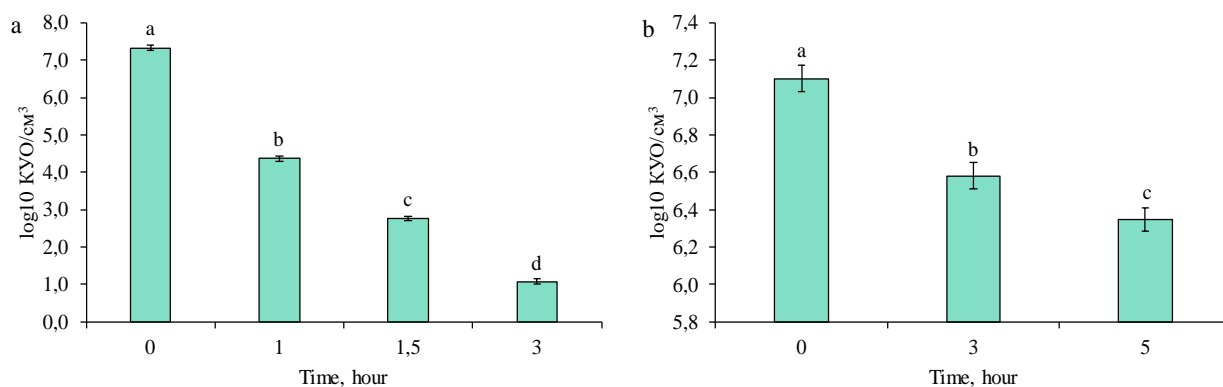


Fig. 2. Effect of stress factors on the number of viable cells of isolate W: a – pH 2.0; b – 0.5 % bile salts

As shown in Figure 2a, under acidic conditions (pH 2.0), a gradual decrease in the number of viable cells of the isolate was observed throughout the incubation period. The initial value was $(7.33 \pm 0.07) \log_{10} \text{CFU/cm}^3$, whereas after 1.5 hours it decreased to $(2.76 \pm 0.06) \log_{10} \text{CFU/cm}^3$, indicating a negative effect and low resistance to acidic pH. After 3 hours, the number of viable cells decreased further to $(1.08 \pm 0.07) \log_{10} \text{CFU/cm}^3$. These results indicate significant inhibition of the viability of isolate W under conditions simulating the gastric environment.

In the presence of bile salts, a less pronounced decrease in cell number was observed. The initial count was $(7.10 \pm 0.03) \log_{10} \text{CFU/cm}^3$, and after 3 hours of incubation, the reduction amounted to 7.3% of the initial value. The final value after 5 hours was $(6.34 \pm 0.05) \log_{10} \text{CFU/cm}^3$. These results indicate high resistance of the isolate to the action of bile salts.

The obtained results are consistent with literature data on the stress resistance of *Lmb. fermentum*. In particular, authors (Panicker et al., 2018) report low resistance of most *Lmb. fermentum* strains to acidic conditions (pH 2.0) and complete loss of viability

after 3 hours of incubation. The most resistant strains showed a decrease in viable cell counts to (2.60 ± 0.2) and $(3.47 \pm 0.7) \log_{10} \text{CFU/cm}^3$, which is in agreement with our findings. Regarding bile salt tolerance, study (Pato et al., 2022) reports the maintenance of high viability of *Lmb. fermentum* cells even after prolonged incubation in a medium containing 0.5 % bile. At the same time, authors (Kim et al., 2022) emphasize the pronounced strain-specific nature of this trait.

One of the important characteristics of LAB with probiotic properties is their ability to inhibit the growth of pathogenic and opportunistic microorganisms. To evaluate the antagonistic activity of isolate W, its effect on the growth of test cultures of bacteria and yeasts was investigated. The results of the antagonistic activity assessment are presented in Table 2.

The isolate exhibited antagonistic activity against the Gram-positive test strains *B. subtilis* UCM B-901 and *S. aureus* UCM B-918, with inhibition zone diameters of $(5.33 \pm 0.31) \text{mm}$ and $(5.23 \pm 0.21) \text{mm}$, respectively. At the same time, no antagonistic activity was observed against Gram-negative strains or yeasts of the genus *Candida*.

These results indicate a selective antagonistic activity of isolate W, primarily directed against Gram-positive microorganisms. The observed

activity requires further investigation using a broader range of Gram-positive test strains.

Table 2.

Antagonistic activity of isolate W against test strains of opportunistic microorganisms

Test strain	Diameter of the inhibition zone, mm
<i>B. subtilis</i> UCM B-901	5,33 ± 0,31 ^a
<i>E. coli</i> UCM B-906	0,00 ± 0,00 ^b
<i>P. aeruginosa</i> UCM B-900	0,00 ± 0,00 ^b
<i>S. aureus</i> UCM B-918	5,23 ± 0,21 ^a
<i>C. albicans</i> UCM Y-1918	0,00 ± 0,00 ^b
<i>C. utilis</i> UCM Y-984	0,00 ± 0,00 ^b

Numerous studies have reported the antagonistic activity of LAB against various opportunistic microorganisms. Study (Sharafi et al., 2015) describes antagonistic activity of *Lmb. fermentum* strains against *B. subtilis*, *E. coli*, *P. aeruginosa*, and *S. aureus*. Authors (Hutt et al., 2006) also report high antagonistic activity of *L. fermentum* ME-3 strain against *E. coli* strains. In contrast to these findings, isolate W exhibited antagonistic activity only against Gram-positive bacteria, which may indicate strain-specificity of the antagonistic properties of *Lmb. fermentum* and a potential dependence on the origin of the strain. The absence of antagonistic activity against *Candida* spp. is consistent with known data on the strain-specific nature of this trait reported in studies (Itapary dos Santos et al., 2019; Yocheva et al., 2024), as well as with the origin of isolate W.

Another important characteristic is antibiotic susceptibility, which allows assessment of the safety of using a particular strain. To determine the antibiotic susceptibility profile of isolate W, a disk diffusion assay was performed. The results are presented in Table 3.

The obtained results indicate that isolate W exhibits both susceptibility and resistance to different groups of antibiotics, which is typical for representatives of LAB. In particular, susceptibility to β-lactam antibiotics (ampicillin, amoxicillin/clavulanic acid), macrolides, tetracycline, rifampicin, and cephalosporins was observed, which is consistent with literature data for *Lmb. fermentum*. At the same time, isolate W demonstrated resistance to aminoglycosides, vancomycin, polypeptide antibiotics, and quinolones, which may be associated with intrinsic resistance characteristic of LAB. It is known that members of the family *Lactobacillaceae* often exhibit natural resistance to aminoglycosides and vancomycin, which is not associated with the presence of transferable resistance genes (Dec et al., 2025; Georgieva et al., 2015). Thus, the obtained antibiotic susceptibility profile of isolate W

generally corresponds to the characteristics of safe probiotic strains; however, further investigation is required to assess the potential for the transfer of resistance genes.

Table 3.

Antibiotic susceptibility profile of isolate W

Antibiotic group	Antibiotic	Susceptibility of isolate (zone of inhibition diameter, mm)
Penicillins	PEN	R (14)
	AMP	S (16)
	AMC	S (22)
Aminoglycosides	STR	R (0)
	GEN	R (10)
	KAN	R (0)
Amphenicols	CMP	S (30)
Macrolides	ERY	S (21)
	AZY	nd (20)
Tetracyclines	TET	S (20)
Glycopeptides	VAN	R (0)
Polypeptides	BAC	R (15)
Polymyxins	POL	R (0)
Rifampicins	RIF	S (33)
Lincosamides	CLI	nd (39)
Cephalosporins	CEF	S (28)
Quinolones	OFL	R (11)

Note. S – sensitive, R – resistant, 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.

Another important characteristic of probiotic microorganisms is their ability to autoaggregate, which determines their potential for adhesion to the intestinal epithelium and colonization of the gastrointestinal tract. To evaluate this property, the autoaggregation ability of isolate W was studied. The results are presented in Figure 3.

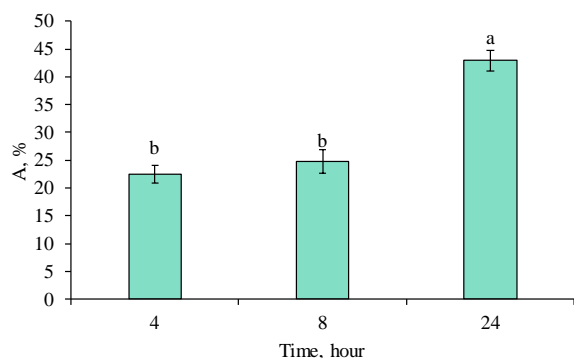


Fig. 3. Changes in the autoaggregation of isolate W during incubation

The isolate was characterized by a gradual increase in autoaggregation over the incubation period. After 4 hours, the value was (22.51 ± 1.57) %, while after 8 hours no statistically significant changes were observed. However, after 24 hours of incubation, the value increased to (42.93 ± 1.89) %. These results indicate that the isolate possesses the ability to autoaggregate, which is an important prerequisite for its adhesive properties.

It is known that autoaggregation levels of LAB may vary significantly depending on the species and strain. In particular, study (Collado et al., 2008) reports an autoaggregation value of (65.9 ± 6.6) % for *L. fermentum* ME-3, corresponding to a high level of this parameter. Studies (Melo et al., 2017) and (Prakash et al., 2021) similarly demonstrate higher autoaggregation values for *Lmb. fermentum*

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strains. Thus, the autoaggregation level determined in this study can be considered moderate compared to literature data for *Lmb. fermentum* strains; however, it indicates the presence of adhesive potential, which requires further investigation.

Conclusions. A lactic acid bacteria isolate was obtained and identified from fermented watermelon juice and, based on the combined physiological-biochemical characteristics and the results of ABIS online analysis, was assigned to the species *Limosilactobacillus fermentum*. The isolate exhibited low resistance to acidic conditions (pH 2.0) and high resistance to bile salts, indicating partial resistance to the conditions of the gastrointestinal tract. Selective antagonistic activity against Gram-positive microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*) was observed, with no activity against Gram-negative bacteria or yeasts of the genus *Candida*. A typical antibiotic susceptibility profile for lactic acid bacteria was determined, along with a moderate autoaggregation ability (up to 42.93 %). The obtained results indicate the potential of the *Lmb. fermentum* isolate as a probiotic strain and justify the need for its further investigation.

Conflict of interest. The authors declare that the study was conducted in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest..

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ФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА МОЛОЧНОКИСЛИХ БАКТЕРІЙ, ІЗОЛЬОВАНИХ ІЗ ФЕРМЕНТОВАНОГО СОКУ КАВУНА

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Ферментовані рослинні продукти є важливим джерелом молочнокислих бактерій (МКБ), проте більшість досліджень зосереджена на традиційних субстратах, тоді як спонтанно ферментовані фруктові соки, зокрема сік кавуна, залишаються малодослідженими. Обмеженість даних щодо видового складу та пробіотичних властивостей молочнокислих бактерій, ізольованих із таких субстратів, визначає необхідність їх подальшого дослідження. Метою роботи є виділення, ідентифікація та оцінка пробіотичних властивостей ізоляту МКБ, виділеного із ферментованого соку кавуна. У роботі використано стандартні мікробіологічні та фізіолого-біохімічні методи дослідження. Виділення та культивування проводили на середовищі MRS при 37 °С. Ідентифікацію здійснювали за морфологічними та фізіолого-біохімічними ознаками з використанням визначника Бергі та програмного забезпечення ABIS online. Стресостійкість оцінювали за виживаністю клітин у середовищі з рН 2,0 та у присутності 0,5 % жовчних солей. Антагоністичну активність визначали методом агар-дифузії, антибіотикочутливість – диск-дифузійним методом, автоагрегаційні властивості – за зміною оптичної густини клітинної суспензії. Із ферментованого соку кавуна виділено ізолят молочнокислих бактерій, який за сукупністю морфологічних та фізіолого-біохімічних ознак ідентифіковано як *Limosilactobacillus fermentum*. Встановлено низьку стійкість до кислого середовища (рН 2,0) та високу стійкість до жовчних солей (0,5 %). Ізолят проявляв антагоністичну активність щодо грампозитивних бактерій (*Bacillus subtilis*, *Staphylococcus aureus*) та не проявляв активності щодо грамнегативних мікроорганізмів і дріжджів роду *Candida*. Визначено типовий профіль антибіотикочутливості та наявність помірної автоагрегаційної здатності (до 42,93 %). Отримані результати можуть бути використані для подальшого дослідження пробіотичних властивостей штаму *Lmb. fermentum*, а також при розробці функціональних ферментованих напоїв і біотехнологічних препаратів.

Ключові слова: молочнокислі бактерії, ферментована рослинна сировина, пробіотичні властивості, ферментований сік кавуна, біотехнологія

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