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NEW LACTIC ACID BACTERIAL STRAINS FROM TRADITIONAL FERMENTED FOODS - BIOPROTECTIVE AND PROBIOTIC POTENTIAL

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ABSTRACT

In traditionally fermented products, lactic acid bacteria (LAB) strains are related to the organoleptic qualities of the products and their beneficial properties. The present study aims to isolate new strains of LAB from the unique and unstudied microbiota of traditional fermented foods artisanal prepared in the Gora region, Albania, and assesses their potential as antagonists of food-associated microorganisms and as probiotics. Different types of fermented food products (yogurt, white-brined cheese, traditional yellow cheese, dried sausage, and spontaneously fermented fruits) were collected and 12 new isolates were obtained. The new isolates' species identification was performed by morphological, physiological characteristics, and 16 S rDNA gene sequencing. The antibacterial activity of the newly isolated strains was performed and antagonistic effects were shown against E. coli (100 %), B. subtilis (100 %), B. cereus (66.67 %,) and P. aeruginosa (25 %). The antifungal activity is a promising advantage, as all of the strains have shown an established antagonistic effect against A. niger (100 %), A. flavus (91.67 %), F. proliferatum (100 %), and P. claviforme (100 %). They have shown strain-specific aggregation (auto-aggregation, co-aggregation), cell surface hydrophobicity, and mucin adhesive abilities. Strains have the capabilities to grow during the stress conditions like low pH, pepsin, pancreatic enzymes, and bile salts, and survival capabilities were at high levels.

Keywords: *lactic acid bacteria, bioprotection, aggregation, GIT resistance, mucin binding.*

INTRODUCTION

People, as consumers, are increasingly turning to the use of functional fermented foods, in which the traditional sensory characteristics of the products are preserved. In addition to nutritional components, these foods have beneficial properties that favor human health [1]. This is one of the reasons for the ever-increasing interest of scientists and industry in researching new strains of microorganisms that are isolated from the microbiota of homemade or artisanal traditional fermented foods and determining their beneficial properties [2]. The studying of new strains of lactic acid bacteria (LAB) is important for the food industry, which is looking at increasing the quality of the products and helping the health of consumers [3, 4]. LAB are very promising sources for new product development, especially those that can meet the growing needs of consumers for natural products and functional foods. Due to the Generally Recognized as Safe (GRAS) status and fulfillment of the criteria of Qualified Presumption of Safety (QPS) in Europe a spectrum of LAB strains has the potential to be applied in a wide range of products of agro-food industries, as dairy products, meat products, fermented cereals and also in cosmetics and pharmaceuticals industries [5, 6]. The use of LAB in the production of different products gives additional taste and aroma and preservative properties [7]. There are many studies that show that LAB influences the final formulation of the product [8]. During the production of lactic acid, they acidify the food and a series of chemical reactions take

place. According to Choi et al., different concentrations of metabolites were present in the product depending on the LAB starter cultures used [9]. As part of the starter cultures, the lactobacilli strains synthesize volatile and nonvolatile compounds that impart distinctive organoleptic properties [10]. A very important role of different LAB strains is their use for the protection against food spoilage microorganisms. This ancient yet modern approach enables the preservation of food in a natural way. The use of LAB as biopreservatives provides the shelf life of food and ensures the safety and quality of food products. They can produce antimicrobial compounds such as organic acids (lactic acid, acetic acid), bacteriocins, and other metabolites, preventing food spoilage and the proliferation of pathogens [11 - 15]. In addition, many strains of LAB have been shown to have a number of probiotic properties, helping to improve the functions of the gastrointestinal tract (GIT), acting as immunomodulators, therapeutics for regulating the microbiota of the GIT, etc. [16]. All these characteristics together increase the need for research for the isolation of new strains and their characterization, in order to increase the quality of functional products by getting as close as possible to traditional food products with their specific characteristics, but at the same time helping in human health.

Gora region is a geographical area in the northeastern part of Albania and the southern part of Kosovo. The area is inhabited by people called Gorani. This area, due to its geographic extent and climatic conditions, favors the development of agriculture. The local people mainly deal with the production of dairy products such as yogurt, cheese, and yellow cheese; meat (sausage, pastrami); fermented vegetables and fruits; brandy; honey, etc. These homemade and artisanal products are processed in traditional conditions according to the methods inherited from their ancestors. Fermented products from these territories are characteristic, with specific tastes and beneficial effects, with rich microbiota not previously studied.

Thus, the current study aimed to isolate new strains of lactic acid bacteria from several types of artisanal fermented food products traditional for the Gora region and unstudied before. The newly isolated strains were taxonomically identified, and the bioprotective properties as antibacterial and antifungal activities and the probiotic potential were studied.

EXPERIMENTAL

Samples

Samples of fermented foods from the Gora region of the Balkan peninsula were collected, characterized by traditional to extensive agriculture in the local population for home production of fermented products. Samples were selected from three main groups of homemade (artisanal) products, prepared by traditional technologies: a) fermented dairy products (yogurt, white cheese, yellow cheese); b) raw-dried meat products (sausage); c) spontaneously fermented fruits. One gram or 1 mL was enriched in a de Man Rogosa, and Sharpe (MRS) broth (Merck, Germany) and incubated at 41ºC, 37ºC, and 30ºC for 24 h, in order to isolate mesophilic and thermophilic LAB strains. Single colonies are selected for the isolation of lactic acid bacteria.

Determination of phenotypic, biochemical characteristics and identification of isolated strains

Morphological characteristics of newly isolated strains were observed using the microscope method and gram staining following the manufacturer's Gram Staining Kit (Sigma-Aldrich, USA) protocol. Determination of catalase activity was performed according to Duke et al. [17] with 3 % H_2O_2 on the microscope slide, determination of oxidase activity was performed using Oxidase Strips (Sigma Aldrich, USA) and determination of peroxidase activity was performed using Quantofix® Peroxide 100 strips for strains cultivated in MRS broth (Merck, Germany) and 10 % skimmed milk (SM) (Himedia, India). Determination of the coagulation capacity of newly isolated strains was in 10 % skimmed milk (Himedia, India). The presence of coagulation was monitored between the 16^{th} h to the 24^{th} h. The biochemical profile was determined using API® 50CHL kit (BioMerieux, France), and results from the tests were analyzed by the APIWEBTM software and compared with the database.

The DNA of isolated strains was extracted and purified using Zymo Research Quick-DNA Miniprep Plus Kit, according to the instructions of the manufacturer. The isolated DNA molecules from each strain were subjected to a PCR reaction to amplify the 16S rDNA gene. The reaction was carried out in PCR tubes with lyophilized GR Healthcare Illustra™ PuReTaq Ready-To-Go PCR Beads using universal primers 27F and 1492R. The

standard PCR protocol was, a pre-denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94ºC for 60 s annealing at 57ºC for 75 s, and extension at 72ºC for 75 s with a final extension at 72ºC for 5 min. PCR products from all strains were sequenced by Macrogen Inc., The Netherlands. The generated sequences were analyzed by comparative sequence analysis (BLASTN) against available sequence data on the National Center for Biotechnology Information (NCBI) database.

Determination of antibacterial activity

Antibacterial activity was determined by the agar-well diffusion method using cell-free supernatants from 24-hour cultures of strains in variants: native cell-free supernatants (CFS) and neutralized cell-free supernatants (NCFS) according to Nikolova et al. [18]. Six test pathogens, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 11778, and pathogenic yeast *Candida albicans* ATCC 18204 were used for antibacterial assays. Overlays of test pathogens (0.5 McFarland) were prepared on agar plates, allowed to dry and wells were made. The 24h culture of the studied strains was centrifuged (6000 x g for 15 min at 10ºC), and the supernatant (CFS or NCFS) was added to the wells and allowed to diffuse. The plates were incubated at the corresponding temperatures of 37ºC, and 30ºC for 24 h. Clear zones around the well confirmed the antibacterial activity and the inhibition zone diameters were expressed in millimeters.

Determination of antifungal activity

Antifungal activity was determined by the agar-layer diffusion method according to Tropcheva et al. [19]. Four test micromycetes species, *Aspergillus niger* A3, *Aspergillus flavus* NBIMCC 916, *Fusarium proliferatum* BT 140 (an isolate), and *Penicillium claviforme* BT 136 were used for antifungal assays. The plates were incubated at 30ºC and the diameters of the growing mold colonies were measured daily until the mold in the control sample filled the Petri dishes. The inhibition effect was calculated following the formula:

percentage of inhibition effect =
= (diameter of the colony under the effect of strains/ diameter of the colony of control) x 100

Auto-aggregation, co-aggregation assays, and cell surface hydrophobicity

Auto-aggregation, co-aggregation, and hydrophobicity assays were carried out according to Tuo et al. with modifications [20]. For the auto-aggregation assay, bacterial culture was centrifuged at 6000 x g for 15 min at 10ºC and standardized to OD = 0.6 ± 0.05 at 600 nm measured with a spectrophotometer. The bacterial suspension was incubated at room temperature and the absorbance was measured at times 0, 3, and 5 h. Auto-aggregation percentage was expressed as: $[1 - A_t/A_0] \times 100$, where A_t represents the absorbance at time t = 3 h or 5 h and A_0 the absorbance at t = 0.

For the co-aggregation assay, an equal volume of bacterial suspension (OD = 0.53 ± 0.05 at 600 nm) prepared as described for auto-aggregation analysis and pathogen strain (*E. coli* ATCC 25922) were mixed. Absorbance measured in spectrophotometer was determined at times 0 and 4 h. Co-aggregation percentage was expressed following the formula according to Colombo et al. [2] as: $[(A_0 - A_t)/A_0] \times 100$, where A_0 represents the absorbance of the mix immediately after mixing and A_t represents the absorbance of the mix at time t = 4 h.

For the hydrophobicity (affinity to hydrocarbons), an equal volume of each LAB strain suspension and xylene were mixed by vortexing for 3 min and incubated at room temperature for 1 h. The absorption of the aqueous phase was measured at 600 nm with a spectrophotometer. Hydrophobicity was expressed as adhesion percentage according to the formula: $[(A_0 - A)/A_0] \times 100$, where A represents the absorbance at time t = 1 h and A_0 the absorbance at t = 0.

In vitro assessment of isolated strains under stimulated conditions of different GIT departments

The acid tolerance, pancreatic enzymes, and bile tolerance assays were performed according to Jatmiko et al. in microwell plates (Corning Incorporated Costar, USA) [21]. MRS broth was adjusted to pH 2 using 1N HCl and add pepsin 1 mg mL^{-1} (Himedia, India). The MRS broth with adjusted pH and pepsin (900 µL) placed in microwell plates was then inoculated with 100 µL of the isolated strains culture. The plates were incubated at 37ºC for 3 h and the absorbance at 600 nm was measured in SPECTROstar® Nano Microplate Reader (BMG LABTECH, Germany). Growth in MRS

broth without adjusted pH and pepsin served as control. Pancreatic enzyme tolerance of isolated strains was performed in the same way as acid tolerance using pancreatin 1 mg/mL (Himedia, India) and incubated for 24 h. Growth in MRS without pancreatin served as control. The bile salt tolerance of isolated strains was conducted using MRS broth supplemented with three bile salt concentrations of 0.1 %, 0.3 %, and 1.0 % (Sigma-Aldrich, Italy) and incubated for 24 h. Growth in the MRS broth without bile salts served as a control.

The Coefficient of inhibition (C_{inh}) was calculated according to Sharifi Yazdi et al. [22] as follows: $C_{inh} = (\Delta OD_{control} - \Delta OD_{treatment})/\Delta OD_{control}$. ΔOD was calculated $OD_t - OD_0$, where OD_0 is the absorbance at time 0 and OD_t is the absorbance at time t. The $C_{inh} \leq 0.40$ was considered significant, for the isolates to be considered a suitable probiotic candidate [23].

Strain growth was measured according to Missotten et al. [24], as: $OD_t - OD_0$, where OD_t is the absorbance at 600 nm after the time of incubation, and OD_0 is the absorbance at the start.

Survival capability of isolated strains in acid conditions and bile salts

The newly isolated strains were tested for survival capability according to Simões et al. with modifications [25]. For the survival of isolated strains in acid conditions, they were cultured in 900 µL MRS broth with and without adjusting pH to 2 and adding pepsin and incubated at the corresponding temperature for 3 h. For the survival of isolated strains in bile salts, the strains were cultured in MRS broth with and without containing 0.3 % (w/v) bile salt (Sigma-Aldrich, Italy) and incubated for 24 h. Viable bacteria were counted by plating serial dilutions on MRS agar. The percentage survival of the bacteria was calculated as follows:

Survival % = [final (log CFU mL^{-1}) / control (log CFU mL^{-1})] * 100.

The pH was measured after the strain cultivation in MRS broth.

Adherence ability of isolated strains to mucin

The method of adherence to mucin was applied according to Monteiro et al. [26] with modification at the final suspension of LAB standardized by spectrophotometry SPECTROstar® Nano Microplate Reader (BMG LABTECH, Germany) to OD 600 nm = 0.35 ± 0.05. An amount of 100 µL (10 mg/mL) mucin (Sigma-Aldrich, USA) solution in PBS was added to the walls of microplates (Corning Incorporated Costar, USA), and incubated overnight at 4ºC. The next day, the wells were washed twice with 200 µL PBS. Then, were saturated with a 2 % bovine serum albumin (BSA) solution (SERVA, New York) for 4 h at 4ºC and washed again twice with 200 µL PBS. Bacteria cultures were washed three times in PBS and re-suspended at absorbance until 0.35 ± 0.05 at OD 600 nm. Aliquots of 100 µL of bacterial suspension were added to the wells of the microplate in three replicates for each strain and microplates were incubated at 37ºC for 1 h. Then to remove non-adherent bacteria, the wells were washed 10 times with 100 µL PBS each time. The wells were treated with 200 µL of 0.5 % Triton X-100 (Sigma-Aldrich, USA) and microplates were incubated under orbital shaking at 100 rpm at 23ºC for 2 h. The number of bacteria with the binding ability to mucin was estimated by serial dilution in PBS and planting on MRS agar, followed by incubation at 37ºC under anaerobic conditions for 48 h. As negative control was used mucin-containing wells without bacteria.

Statistical analysis

All experiments were performed in triplicate. Results are presented as mean ± standard deviations (SD). Statistical analyses were performed using SPSS 19.0 for Windows statistical software. The statistical comparisons between the isolates were performed by the Pearson correlation test with a significance of $p < 0.05$ for comparing the survival capability of strains. Pairwise correlations among mucin binding ability, auto-aggregation, co-aggregation, and hydrophobicity were determined using Pearson's correlation coefficient analysis at a $p \leq 0.05$. Paired Samples T-test was used for the relation between the coefficient of inhibition and the growth of bacteria during stress conditions.

RESULTS AND DISCUSSION

Isolation of new LAB strains from the microbiota of fermented products

A total of 12 strains of LAB were isolated from homemade cow and goat yogurt, cow and goat white-brined cheese, sheep yellow cheese, cow raw-dried meat products, and spontaneously fermented fruits. All newly isolated strains grow well on an elective MRS medium.

Determination of phenotypic, biochemical characteristics and identification of isolated strains

In 2020, was proposed a new classification of the family *Lactobacillaceae*, which includes an updated genus *Lactobacillus* (based on the *Lactobacillus delbrueckii* group*), Paralactobacillus, Pediococcus* and 23 new genera consisting of species previously assigned to the previous genus *Lactobacillus* [27]. Zheng et al. offer the generic term '*lactobacilli'* to continue to be used to designate all microorganisms, classified as *Lactobacillaceae* until 2020 [27]. All newly isolated strains are gram-positive, catalase, and oxidase-negative (Table 1). Isolates with rod-shaped morphology predominate (91.67 %), but one has coccoid morphology (8.33 %). Some of them are thermophiles and others are mesophiles. Of all the newly isolated strains, 7 were identified as belonging to the species *Lactiplantibacillus plantarum*. *Lactiplantibacillus plantarum* is one of the species of lactobacilli that are often found in various spontaneously and traditionally prepared fermented products and the identification of seven of the newly isolated strains of this species is an expected result [28]. Two strains KZM 2-11-1 and KZM 2-11-3, isolated from goat yogurt were identified as belonging to Lactobacillus delbrueckii ssp. bulgaricus. Isolation and identification of strains from the species *Lactobacillus delbrueckii* ssp. *bulgaricus* is also an expected result for samples of homemade yogurt, but the different strains contribute to the specific properties of the traditional product including texture and consistency, the development of the aroma, flavor, and color [29]. One strain (KO 3-7-5) was isolated from yellow cheese and identified as the species *Loigolactobacillus coryniformis*. The strains of this species have been isolated from goat's milk products, but they were detected at a low frequency in fermented foods [30, 31]. The strain *L. coryniformis* CECT 5711 was defined as an attractive potential probiotic strain [30] and this directed research interest to the newly isolated strain KO 3-7-5. One of the strains KC 5-13 is defined as *Pediococcus pentosaceus*. This species that are isolated from various fermented foods, the strains from this species are definite as probiotics [32 - 36] and are part of starter cultures for the production of cheese and meat products [37]. One strain, C 10-31-3 is an isolate of raw-dried meat products and is defined as belonging to the species *Latilactobacillus sakei*. This species is traditionally isolated from rice wine [38] and fermented fish and meat products [39]. Strains of this species are included as part of starter cultures for the production of raw-dried [40], and smoked meat products [41], where they are also important as bioprotective agents against pathogenic microflora [42]. Different species have been isolated in similar products in different countries of the world, mentioning species such as *L. plantarum, L. delbrueckii* ssp. *lactis, L. delbrueckii* ssp. *bulgaricus, L. coryniformis, Leuconoctoc mesenteroides, L. brevis, L. fermentum, L. kefiri, S. thermophilus, P. pentosaceus, P. acidolactici* and *L. sakei* [21, 22, 31, 32, 35, 36, 39, 43].

LAB can produce hydrogen peroxide and this is associated with their bio-protective potential [44]. A screening test for H_2O_2 production from the studied strains was also performed and the obtained results are presented in Table 1. The H_2O_2 production was detected only for two strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* when culturing on SM medium. The coagulation ability of newly isolated strains was determined in SM medium for 24 h. Coagulation was observed until 16th h of incubation for 11 of the studied strains, but the strain *Latilactobacillus sakei* C 10-31-1 can not coagulate SM even after 24 h.

Determination of antimicrobial activity

Table 2 presents the results of the antimicrobial activity of the isolated strains. In the studied strains, we have found antibacterial activity against both types of pathogens gram-positive and gram-negative. All strains inhibit the growth of *E. coli* and *B. subtilis.* The strains *L. plantarum* KBB 7-1 and KBB 11 have shown the highest antagonistic activity, followed by *L. delbrueckii ssp. bulgaricus* KZM 2-11-1 and KZM 2-11-3. Eight of the strains *L. plantarum* KO 4-4, KC 5-12, KC 5-14, KZC 8-21-1, KZC 8-23-5, KBB 7-1, KBB 11, and *P. pentosaceus* KC 5-13 have shown antagonistic activity against *B. cereus*, and three of the strains belonging to *L. plantarum* KO 4-4, KBB 7-1, KBB 11 have shown antagonistic activity against *P. aeruginosa.* The results for the antibacterial activity of LAB strains from different species against different pathogens were described in scientific publications [6, 14, 21, 32, 36, 45, 46]. This shows that the newly isolated strains have a good bioprotective potential for the inhibition of bacterial pathogens.

Antimicrobial agents produced by lactic acid bacteria as lactic acid, and acetic acid are bioactive at low pH and can act synergistically [47]. In neutralized cell-free supernatant (NCFS) variant there was no activity

Table 1. Characterization of 12 newly isolated strains of fermented products prepared by traditional technology.

									Species identification	
Strains	Origin	Cell morphology	Gram	Oxidase	Catalase	Peroxidase MRS	Peroxidase SM	Coagulation	API WEB	16S rDNA gene sequence
KZM 2-11-1	Goat yogurt	Rod-shaped	+	-	-	-	+	+	*Lactobacillus delbrueckii* ssp. *bulgaricus*	*Lactobacillus delbrueckii* ssp. *bulgaricus*
KZM 2-11-3	Goat yogurt	Rod-shaped	+	-	-	-	+	+	*Lactobacillus delbrueckii* ssp. *bulgaricus*	*Lactobacillus delbrueckii* ssp. *bulgaricus*
KO 3-7-5	Sheep yellow cheese	Rod-shaped	+	-	-	-	-	+	Not identified	*Loigolactibacillus coryniformis*
KO 4-4	Sheep yellow cheese	Rod-shaped	+	-	-	-	-	+	Not identified	*Lactiplantibacillus plantarum*
KC 5-12	Cow white cheese	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*
KC 5-13	Cow white cheese	Coccoidal	+	-	-	-	-	+	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*
KC 5-14	Cow white cheese	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*
KZC 8-21-1	Goat white cheese	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*
KZC 8-23-5	Goat white cheese	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*
C 10-31-3	Cow dried meat	Rod-shaped	+	-	-	-	-	-	Not identified	*Latilactibacillus sakei*
KBB 7-1	Fermented fruit	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*
KBB 11	Fermented fruit	Rod-shaped	+	-	-	-	-	+	*Lactiplantibacillus plantarum*	*Lactiplantibacillus plantarum*

Activity: + is for strains gram-positive, the presence of peroxidase, and coagulation; - is for strains gram-negative and no presence of (catalase, oxidase, peroxidase activity, or coagulation).

Table 2. Antimicrobial activity of CFS from newly isolated LAB strains.

	Test pathogens					
Strains	*E. coli*	*P. aeruginosa*	*B. subtilis*	*B. cereus*	*S. aureus*	*C. albicans*
L. bulgaricus KZM 2-11-1	12.5 ± 0.50	-	12.5 ± 0.01	-	-	-
L. bulgaricus KZM 2-11-3	13.3 ± 0.25	-	13.0 ± 0.00	-	-	-
L. coryniformis KO 3-7-5	12.3 ± 0.75	-	11.3 ± 0.03	-	-	-
L. plantarum KO 4-4	11.5 ± 0.25	18.0 ± 0.82	11.0 ± 0.00	16.0 ± 0.82	-	-
L. plantarum KC 5-12	12.3 ± 0.25	-	12.0 ± 0.02	12.3 ± 0.47	-	-
P. pentosaceus KC 5-13	12.5 ± 0.50	-	11.8 ± 0.25	13.5 ± 0.50	-	-
L. plantarum KC 5-14	11.0 ± 0.01	-	12.0 ± 0.00	15.7 ± 0.47	-	-
L. plantarum KZC 8-21-1	12.3 ± 0.25	-	11.3 ± 0.25	15.0 ± 0.82	-	-
L. plantarum KZC 8-23-5	12.3 ± 0.75	-	12.0 ± 0.00	15.3 ± 1.24	-	-
L. sakei C 10-31-3	11.5 ± 0.04	-	11.0 ± 0.00	-	-	-
L. plantarum KBB 7-1	17.5 ± 0.25	16.5 ± 0.50	18.5 ± 0.50	12.75 ± 0.25	-	-
L. plantarum KBB 11	17.0 ± 0.01	16.5 ± 0.50	19.0 ± 0.02	14.25 ± 0.25	-	-

The inhibition zone diameters were expressed in millimeters, as mean ± SD in triplicate; - is not determined the inhibition effect.

recorded against test pathogens, and it could be assumed that the activity found in the native cell-free supernatant (CFS) variant was mainly due to the production of low molecular mass organic acids [46, 48]. No antagonistic activity was detected for *C. albicans*.

Antagonistic activity of isolated strains against filamentous moulds

The selected test molds are of species that are associated with contamination of the food chain and can contribute to the development of health problems in consumers. Despite the increased acidity, fermented dairy products, as well as other fermented foods, are at risk of contamination with pathogenic, toxigenic, deteriorative or allergenic fungi from the genera as *Aspergillus*, *Fusarium*, *Penicillium* [19]. The results obtained for the antifungal activity of LAB isolates from fermented food products are presented in Fig. 1. All tested strains completely inhibited the growth of two of the test moulds *F. proliferatum* and *P. claviforme*. It is important to note that two newly isolated strains *L. delbrueckii ssp. bulgaricus* suppress moulds in species such as *Fusarium* and *Penicillium*. In most strains of *L. plantarum*, a well-defined inhibitory effect against the genus *Aspergillus* was observed. The growth of *A. niger* is completely suppressed by four of the isolated strains belonging to *L. plantarum* and the growth of *A. flavus* was completely suppressed by nine strains, all seven of *L. plantarum*, *L. coryniformis,* and *P. pentosaceus*. The established antifungal activity of the newly isolated strains is a promising advantage, which suggests their potential applications as natural food preservatives in various food technologies [11]. Numerous studies have shown that strains from species *L. plantarum, P. pentosaceus, L. sakei,* and *L. coryniformis* have shown inhibitory effects on a wide range of pathogens including *Aspergillus sp.*, *Penicillium sp.,* and *Fusarium sp.* [6, 12, 19, 49].

Auto-aggregation, co-aggregation, cell surface hydrophobicity assays

Bacteria can form aggregates through auto-aggregation (accumulation of the same type of bacteria) and co-aggregation (accumulation of different bacterial species). Probiotic bacteria, to achieve the desired benefits, must form a large mass through accumulation [51]. Lactobacillus species, protect from pathogen colonization, forming a barrier population by mechanisms that include auto-aggregation, co-aggregation, and adhesion to epithelial surfaces [44]. In this study, all isolated strains were determined for their ability to auto-aggregate, co-aggregate, and

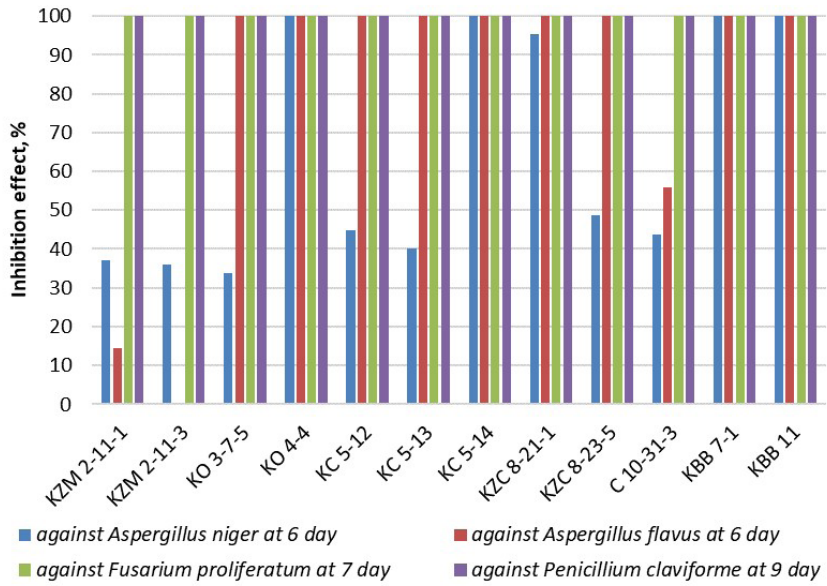

Fig. 1. Antifungal activity of newly isolated strains from dairy, meat, and fermented fruit products, presented as a percentage of inhibition effect.

affinity to hydrocarbons (hydrophobicity). The results are presented in Table 3. The auto-aggregation ability in isolated strains is strain-dependent. At 3 h of incubation at room temperature, all isolated strains have been shown to exhibit autoaggregation of 9 % - 44.2 %, which increased with time, and at 5 h of incubation reached 11.1 % - 70.1 %. The highest recorded autoaggregation has shown strains belonging to *L. delbrueckii* ssp. *bulgaricus* KZM 2-11-1 and KZM 2-11-3 followed by *L. plantarum* KC 5-12 with 68.0 %, 70.1 %, and 60.6 %, respectively. These strains have auto-aggregation ability, making them interesting candidates for potential probiotic applications. Similar results were found in other studies as they have shown good auto-aggregation ability to strains of *L. plantarum*, and *L. delbrueckii* [44, 52]. Regarding the ability of bacteria to form aggregates with genetically different strains (co-aggregation) is contradictory and is divided into two opposite aspects. On the one hand, bacteria must have low co-aggregation abilities to minimize the colonization of pathogens in the gastrointestinal tract. On the other hand, they must have high co-aggregation abilities, because it is one of the possible ways to eliminate pathogens from the gastrointestinal tract [51]. From the isolated strains, co-aggregation with *E. coli* at 4 h of incubation at room temperature has shown strains *L. plantarum* KBB 11 (15.9 %), *L. sakei* C 10-31-1 (14.4 %), and *L. delbrueckii* ssp. *bulgaricus* KZM 2-11-1 (13.8 %). The results of the other investigators have been shown to be similar. Strains of *L. plantarum*, *L. delbrueckii* ssp. *bulgaricus* have a co-aggregation ability that is strain-dependent [44, 53, 54]. The high hydrophobicity of probiotic strains represents higher interaction with the epithelium cells of the gastrointestinal tract, which indicates the better exclusion of pathogens [25, 47]. The highest hydrophobicity was determined for strains belonging to *L. delbrueckii* ssp. *bulgaricus* KZM 2-11-1 and KZM 2-11-3 at 1 h of incubation at room temperature with 35.5 % and 29.7 %. Similar results were obtained in other studies for high hydrophobicity to *L. delbrueckii* ssp. *bulgaricus* and for low hydrophobicity to *L. plantarum* [44, 55, 56]. Aggregation abilities, hydrophobicity (Table 3), and adhesive abilities to mucin (Fig. 2) were compared for correlation among them using Pearson's correlation (2-tailed) in SPSS 19.0 software. A positive correlation was found between auto-aggregation and hydrophobicity at level $p < 0.01$. In other studies, were found different correlations, e.g., the relative adhesion values of the Lactobacillus strains were positively correlated with both hydrophobicity and aggregation using Spearman's correlation coefficient [57], and the auto-aggregation abilities of the lactobacilli were highly correlated with

Table 3. Percentage (%) of auto-aggregation, co-aggregation, and cell surface hydrophobicity of isolated strains.

Strains	Auto-aggregation		Co-aggregation	Hydrophobicity
	3 h	5 h	4 h	1 h
L. bulgaricus KZM 2-11-1	42.9 ± 8.3	68.0 ± 4.9	13.8 ± 0.8	35.5 ± 5.3
L. bulgaricus KZM 2-11-3	44.2 ± 15.0	70.1 ± 7.3	11.9 ± 4.4	29.7 ± 8.0
L. coryniformis KO 3-7-5	22.6 ± 1.6	44.2 ± 6.1	6.4 ± 1.0	15.6 ± 0.4
L. plantarum KO 4-4	11.3 ± 0.5	17.5 ± 3.0	7.4 ± 0.7	1.2 ± 0.1
L. plantarum KC 5-12	37.3 ± 1.5	60.6 ± 4.4	9.9 ± 0.2	9.9 ± 4.9
P. pentosaceus KC 5-13	17.5 ± 1.9	35.1 ± 3.9	8.7 ± 1.1	16.8 ± 5.8
L. plantarum KC 5-14	19.6 ± 0.3	29.0 ± 5.5	7.8 ± 1.7	29.5 ± 0.7
L. plantarum KZC 8-21-1	16.3 ± 1.4	29.0 ± 7.0	11.5 ± 1.0	5.1 ± 2.6
L. plantarum KZC 8-23-5	12.1 ± 0.4	18.5 ± 1.9	7.2 ± 0.7	6.9 ± 2.8
L. sakei C 10-31-3	21.2 ± 0.2	32.9 ± 3.5	14.4 ± 0.8	2.7 ± 0.0
L. plantarum KBB 7-1	9.0 ± 0.5	11.1 ± 0.1	10.6 ± 0.1	3.2 ± 2.4
L. plantarum KBB 11	16.2 ± 0.7	18.6 ± 0.4	15.9 ± 0.2	0.8 ± 0.6

Data are expressed as percentages calculated following the formulas above of auto-aggregation measured after 3 and 5 h, co-aggregation after 4 h, and hydrophobicity after 1 h of incubation. The values are means of triplicate measurements ± SD. A Pearson correlation (2-tailed) was significant between auto-aggregation and hydrophobicity at a level of $p < 0.01$.

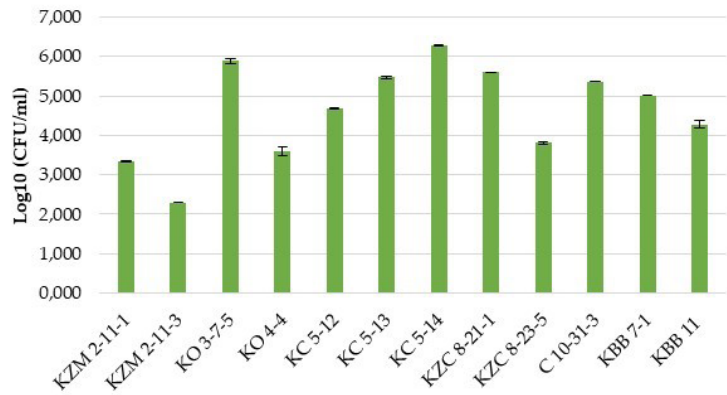

Fig. 2. Mucin binding ability of newly isolated strains. Data are expressed as Log10 (CFU mL^{-1}) ± SD.

their co-aggregation to different pathogens [58].

In vitro assessment of the growth of newly isolated strains under simulated conditions of different gastrointestinal tract departments

In this study, isolated strains are exposed to different conditions to determine the bacteria growth during the stress conditions. Low pH, gastric enzymes, and bile salts are examples of barriers to the gastrointestinal tract that probiotic bacteria need to resist after being ingested [59]. Bacteria pass through the digestive system from the mouth to the stomach and then to the intestine. The transit time of food in the stomach is 15 min - 4 h where the pH is 1.5 to 2 and pepsin is present, an enzyme that breaks proteins into smaller peptides. Pepsin, produced in the stomach it is one of the main digestive enzymes and is active at low pH between 1.5 - 2. After the stomach, food digestion goes to the small intestine where

the pH is 6.5 - 7, and the pancreatic enzymes are present, and then to the large intestine [60]. These enzymes break down sugars, fats, and starches. The bile salts are excreted into the intestine and play a crucial role in digestion (transit time 12 - 24 h). Intestinal bacteria use bile salts as environmental signals and in certain cases as nutrients and electron receptors. However, bile salts can disrupt bacterial membranes, denature proteins, and cause oxidative DNA damage [61].

The coefficient of inhibition was investigated and the results are presented in Table 4. The coefficient of inhibition varies between strains in acidic conditions. The lowest coefficient of inhibition is for strain *L. delbrueckii* ssp. *bulgaricus* KZM 2-11-1, and *L. delbrueckii* ssp. *bulgaricus* KZM 2-11-3. All strains have shown that were not affected by pancreatin, or there was a very low inhibition. Strains exposed to three different concentrations of bile salts (0.1 %, 0.3 %, 1.0 %), have shown results as below. In 0.1 % bile salts, five strains (*L. plantarum* KO 4 - 4, *L. plantarum* KC 5 - 12, *P. pentosaceus* KC 5-13, *L. plantarum* KZC 8-21-1 and *L. plantarum* KZC 8-23-5) have a significant C_{inh}. At concentration 0.3 % bile salts, three strains (*L. plantarum* KC 5-12, *P. pentosaceus* KC 5-13, and *L. plantarum* KZC 8-23-5) have a C_{inh} less than 0.4, while at concentrations 1.0 % only one strain *L. plantarum* KC 5-12 have a C_{inh} close to 0.4. Similar results were found in other studies. Strains of different species including *L. plantarum* and *P. pentosaceus* are able to survive under simulated conditions of different gastrointestinal tract departments [22, 23, 62, 67]. LAB are acidophilic, which means they are tolerant to low pH. However, this needs to be differentiated from a condition of high concentration of free acids (H+), because the free acids may cause growth inhibition [59]. Changes in the external environment such as pH and bile salts can cause metabolic disorders, even cell death. Microbial cells suffer acidic, and other stresses but resistance mechanisms have evolved in microorganisms to survive in stress conditions. The intracellular pH decreases with the increase of acidity in the environment, causing the energy consumption of the cells to keep their metabolic activity under control. When pH drops too low, pH homeostasis is disrupted, causing protein and DNA damage and cells to shrivel [61, 63]. For this reason, the growth rate of the strains was evaluated, to see,

Table 4. Coefficient of inhibition of newly isolated strains at acidic conditions, pancreatic enzymes, and bile salts.

Coefficient of inhibition	3 h	24 h	24 h		
Strains	Pepsin (1 mg mL^{-1}) +pH2	Pancreatin (1 mg mL^{-1})	Bile 0.1 %	Bile 0.3 %	Bile 1.0 %
L. bulgaricus KZM 2-11-1	0.70 ± 0.19	0.17 ± 0.12	0.72 ± 0.33	1.09 ± 0.09	1.12 ± 0.08
L. bulgaricus KZM 2-11-3	0.60 ± 0.23	-1.12 ± 0.36	1.04 ± 0.06	1.26 ± 0.04	1.20 ± 0.08
L. coryniformis KO 3-7-5	0.97 ± 0.03	0.17 ± 0.05	0.68 ± 0.04	1.04 ± 0.07	1.11 ± 0.06
L. plantarum KO 4-4	0.93 ± 0.59	-0.04 ± 0.02	0.10 ± 0.06	0.69 ± 0.09	0.94 ± 0.37
L. plantarum KC 5-12	**0.93 ± 0.32**	**-0.01 ± 0.01**	**-0.05 ± 0.29**	**0.31 ± 0.13**	**0.45 ± 0.30**
P. pentosaceus KC 5-13	**1.01 ± 0.05**	**0.001 ± 0.07**	**0.14 ± 0.09**	**0.18 ± 0.05**	**0.69 ± 0.06**
L. plantarum KC 5-14	1.02 ± 0.12	0.51 ± 0.02	0.61 ± 0.05	0.60 ± 0.04	0.52 ± 0.02
L. plantarum KZC 8-21-1	0.97 ± 0.29	0.20 ± 0.04	0.11 ± 0.01	0.58 ± 0.13	0.81 ± 0.49
L. plantarum KZC 8-23-5	**0.94 ± 0.05**	**0.07 ± 0.01**	**0.11 ± 0.04**	**0.39 ± 0.11**	**0.85 ± 0.31**
L. sakei C 10-31-3	1.20 ± 0.12	-0.26 ± 0.18	0.46 ± 0.09	1.04 ± 0.04	1.06 ± 0.04
L. plantarum KBB 7-1	1.20 ± 0.11	0.16 ± 0.01	0.43 ± 0.04	0.95 ± 0.03	1.04 ± 0.07
L. plantarum KBB 11	1.02 ± 0.03	0.26 ± 0.05	0.50 ± 0.06	0.83 ± 0.02	0.97 ± 0.04

The values of the coefficient of inhibition are means of triplicate measurements ± SD. Strains that according to Salehizadeh et al. [24] at C_{inh} less than 0.4 at a concentration of bile salt 0.3 %, considered suitable probiotic candidates are bolded.

if the metabolic disorders prevent cell growth during stress conditions. The growth of bacteria strains was measured as absorbance at 600 nm and according to Missotten et al. [24], expressed as +, growth ≤ 0.2; ++, 0.2 < growth ≤ 0.5; +++, growth > 0.5, and the results are featured in Table 5. It has been shown that some of the strains can grow in acidic conditions. All strains can grow in the presence of pancreatin. The growth rate of bacteria in bile is strain-dependent, and bile salt concentration-dependent. A Paired Samples T-test was made for analyzing the relation between the coefficient of inhibition and the growth of bacteria during the stress conditions and was found Paired Samples correlation with significance ($p < 0.05$).

Survival capability under the influence of stress factors of different departments of the gastrointestinal tract

Microorganisms, to adapt to acidic environments, have improved acid tolerance as a result of metabolic regulatory mechanisms. They improve redox factors for survival, growth, and metabolism by strengthening the glycolytic pathway. Microorganisms can protect or repair macromolecules like DNA and proteins, by specific proteins (DNA polymerase, DNA ligase, chaperones) which are induced by acid stress. Species contain a variety of elements, specific for response to stress factors, and are strain-specific Most microorganisms can adapt to changes in external conditions and can survive [63].

To observe the survival capability of isolated strains under the influence of stress factors of the gastrointestinal tract, strains were exposed to low pH and pepsin, and bile salts with a concentration of 0.3 % as described in the method above. Cell counting was done and viability was calculated. Results are presented in Table 6. After exposure to low pH and pepsin, it was observed that most of the strains were able to survive at a level above 50 %. Only, for three of the strains, *L. bulgaricus* KZM 2-11-1, *L. bulgaricus* KZM 2-11-3, and *L. sakei* C 10-31-3 survival capability was not determined. All strains have a good survival capability in bile salts in intervals from 45 % to 94 %. Several studies showed similar results in the screening for survival capability in acidic conditions and bile salts. Strains of *L. plantarum* and *P. pentosaceus* have good survival capability [22, 23, 62]. Percentages of the viability of strains in both, acid and bile conditions (Table 6) were compared for correlation with the pH of

Table 5. The growth rate of bacteria in low pH and pepsin, pancreatic enzymes, and bile salts in concentrations of 0.1 %, 0.3 %, and 1 %.

	Growth of Bacteria				
Strains	Pepsin (1 mg mL^{-1}) +pH2	Pancreatin (1 mg mL^{-1})	Bile 0.1 %	Bile 0.3 %	Bile 1.0 %
L. bulgaricus KZM 2-11-1	+	++	+	n.d.	n.d.
L. bulgaricus KZM 2-11-3	+	+++	n.d.	n.d.	n.d.
L. coryniformis KO 3-7-5	n.d.	+++	++	n.d.	n.d.
*L. plantaru*m KO 4-*4*	n.d.	+++	+++	++	+
L. plantarum KC 5-12	+	+++	+++	+++	+++
P. pentosaceus KC 5-13	n.d.	+++	+++	+++	+++
L. plantarum KC 5-14	n.d.	+++	+++	+++	+++
L. plantarum KZC 8-21-1	+	+++	+++	+++	++
L. plantarum KZC 8-23-5	+	+++	+++	+++	++
L. sakei C 10-31-3	n.d.	+++	+++	n.d.	n.d.
L. plantarum KBB 7-1	n.d.	+++	+++	+	n.d.
L. plantarum KBB 11	n.d.	+++	+++	++	+

Growth of bacteria: +, growth ≤ 0.2; ++, 0.2 < growth ≤ 0.5; +++, growth > 0.5; n.d.- no growth detected.

Table 6. Survival capability of new strains in acidic and bile conditions.

		Pepsin (1 mg mL^{-1}) +pH2	Bile salt 0.3 %				
LAB strains	pH of strain cultures	Control at 3 h	Pepsin+pH2 at 3 h	Survival (%)	Control at 24 h	Bile salt at 24 h	Survival (%)
L. bulgaricus KZM 2-11-1	3.48	7.73 ± 0.05	n.d.	-	5.50 ± 0.01	2.48 ± 0.35	45
L. bulgaricus KZM 2-11-3	3.50	8.13 ± 0.02	n.d.	-	4.40 ± 0.09	2.56 ± 0.18	58
L. coryniformis KO 3-7-5	3.52	8.25 ± 0.04	5.13 ± 0.01	62	7.91 ± 0.04	4.21 ± 0.00	53
L. plantarum KO 4-4	3.02	8.84 ± 0.03	8.05 ± 0.01	91	8.02 ± 0.02	6.94 ± 0.00	87
L. plantarum KC 5-12	3.19	7.53 ± 0.05	6.09 ± 0.01	81	8.30 ± 0.16	6.91 ± 0.00	83
P. pentosaceus KC 5-13	3.20	8.71 ± 0.01	4.31 ± 0.02	50	8.18 ± 0.06	7.24 ± 0.01	88
L. plantarum KC 5-14	3.03	8.64 ± 0.04	8.09 ± 0.01	94	8.81 ± 0.03	5.74 ± 0.04	65
L. plantarum KZC 8-21-1	2.91	8.33 ± 0.01	7.95 ± 0.00	95	8.39 ± 0.01	6.56 ± 0.07	78
L. plantarum KZC 8-23-5	2.96	8.72 ± 0.02	8.34 ± 0.00	96	8.81 ± 0.10	6.73 ± 0.01	76
L. sakei C 10-31-3	3.84	8.15 ± 0.03	n.d.	-	7.56 ± 0.03	7.12 ± 0.00	94
L. plantarum KBB 7-1	3.84	8.89 ± 0.02	6.22 ± 0.08	70	8.44 ± 0.01	7.13 ± 0.01	84
L. plantarum KBB 11	3.81	8.83 ± 0.01	6.59 ± 0.05	75	8.15 ± 0.07	7.04 ± 0.02	86

The values of log10 (CFU mL^{-1}) are means of measurements ± SD measured after 3 h of incubation for acid and at 24 h of incubation for bile salt. The survival capability data are expressed as % of means. Pearson correlation is significant among pH and survival capability in acid conditions ($p < 0.05$) and no significance was found among pH and survival capability in bile salt ($p > 0.05$); n.d.- no growth detected.

culture cultivated in MRS broth to prove if statistically there is a relationship between the pH level of the culture and their ability to survive in acidic and bile salts conditions. A positive correlation was found between the pH level of culture and viability in acid conditions, using Pearson's correlation coefficient with significance ($p < 0.01$). This shows that the strains that produce higher amounts of lactic acid and have a lower pH in their cultures are able to survive in acidic conditions. Relative tolerance to the final acidic product is strain-dependent. The survival of these bacteria in acidic conditions is related to the control mechanisms of intracellular pH and H^+ and ATPase play the main role [64]. This shows that strains that decrease extracellular pH are better able to survive in acidic conditions of the stomach because they contain more sophisticated mechanisms to protect and recover their cells in such conditions.

Adherence ability of isolated strains to mucin (mucoadhesivity)

The mucus is a special layer found in the gastrointestinal tract (GIT) and plays an essential role in providing lubrication for the passage of food. It participates in cell signaling pathways and protects the host epithelium from commensal microorganisms and pathogens, as well as toxins and other environmental irritants. In the mucus are found glycosylated proteins called mucin. Mucins play a crucial role in physical protection, cell signaling, lubrication, and forming chemical barriers as well as in regulating the concentration and passage of water, ions, and other immune mediators such as antimicrobial peptides and immunoglobulin-A (IgA) within the gut [65]. The adhesion ability of LAB on mucin has attracted attention as one of the main criteria for selecting probiotics, and one of the factors contributing to the persistent beneficial effects of LAB in the intestinal environment [66]. Mucus-binding proteins are a group of proteins that play the role of bacterial surface adhesion and bind to mucin proteins [67]. Many studies have been done on the adhesion abilities of lactic acid bacteria and mucus-binding proteins. A strain of *Lactococcus lactis* (TIL448) has been found to persist a high adhesion ability with pig gastric mucin [68]. Mucus-binding proteins in the adhesion and aggregation properties of *Lactobacillus reuteri* are strain-specific [69]. *Lactobacillus fermentum* BCS87 expresses mucus- and mucin-binding proteins on the cell surface [70]. The adhesion of *Bifidobacterium lactis* Bb12 to a mucus model was more than doubled in the presence of *Lactobacillus* GG or *L. delbrueckii* ssp. *bulgaricus* [71], but there are no documented findings so far that *L. delbrueckii* ssp. *bulgaricus* itself to be bonded to mucin.

In this study, the ability of isolated strains to bind to mucin has been determined. The Log10 (CFU mL^{-1}) of bonded strains to mucin was expressed in Fig. 2. The ability of bacteria to bind to mucin is strain-specific and varies between strains. Some of the strains show very good mucin-binding ability. Strains *L. plantarum* KC 5-14, *L. coryniformis* KO 3-7-5, *L. plantarum* KZC 8-21-1 and *P. pentosaceus* KC 5-13 have shown high binding ability. Cells of strains *L. delbrueckii* ssp. *bulgaricus* was determined to be bonded to mucin at 10^2 to 10^3 CFU mL^{-1}. Although they have a low level of binding with mucin, these two strains show a special characteristic, since these types of strains are not characterized by the ability to bind to the cell surfaces. Mucin-binding ability varies among *L. plantarum* strains showing a wide spectrum of mucin-binding ability, which is strain-specific, from those with a high binding ability such as *L. plantarum* strain KC 5-14, KZC 8-21-1, KBB 7-1, binding by an amount of 10^6-10^5 CFU mL^{-1}, in those with a medium ability such as *L. plantarum* KC 5-12, KBB 11, binding with an amount over 10^4 CFU mL^{-1}, up to those with low ability including here *L. plantarum* KO 4-4 and KZC 8-23-5, with binding ability less than 10^4 CFU mL^{-1}. In other studies, have been found that strains *L. plantarum* and *L. coryniformis* have good binding abilities [21, 67]. No studies have been found that can verify the binding abilities of the *L. sakei* strain with mucus, especially with the mucin protein. Our recorded results have shown a special characteristic of *L. sakei* with a high binding ability of over 10^5 CFU mL^{-1}.

Mucus-binding protein presence was further highly associated with the auto-aggregation of *L. reuteri* strains in washed cell suspensions, suggesting a novel role of this surface protein in cell aggregation [62]. Different factors, like auto-aggregation and the presence of mucin-binding proteins, are correlated with the adhesion abilities of bacteria [72]. From the comparison made between the percentages of auto-aggregation, co-aggregation, hydrophobicity (Table 4), and mucin-binding ability (Fig. 2), no correlation was found between mucin-binding ability, and other abilities (auto-aggregation, co-aggregation, hydrophobicity).

CONCLUSIONS

In this study, the isolated strains have been identified as belonging to the species *L. delbrueckii* ssp. *bulgaricus*, *L. coryniformis*, *L. plantarum*, *L. sakei* and *P. pentosaceus*. Eleven of the twelve strains have good coagulation abilities, and two of them produce H_2O_2. In newly isolated strains were determined antibacterial and antifungal activity, which is a prerequisite for their inclusion as biopreservatives in food products. The strains have antimicrobial activity against Gram-negative and Gram-positive pathogens. Properties determining the probiotic potential of newly isolated strains were also investigated; this is the basis for their inclusion as potential probiotic supplements. Studied LAB strains have shown aggregation ability and have significant adhesive potential, such as mucin binding, which determines their role in the competition of pathogens. Most of the isolated strains can survive at low pH and simulated stomach conditions. They are not affected or are affected at a very low level by the impact of pancreatin. Most of the isolated strains have growth abilities at bile salts and survival capability after the influence of stress factors in the GIT of 65 to 88 %. This shows that most of the studied strains are able to withstand the stress conditions in the GIT departments and suggests their probiotic potential.

In conclusion, the lactic acid bacteria isolated in this study can be used in the production of fermented foods. They can serve as bio-preservatives by inhibiting the growth of food spoilage organisms. Also, they can serve as probiotic supplements in food production. Further studies are needed to determine the potential of the selected strains to be included as starter cultures in fermented products. Also, it is necessary to study the role of selected strains as bio-preservatives by co-culturing with pathogens and identifying compounds that inhibit pathogens. Such studies will contribute to determining the technological applicability of newly isolated LAB strains in new safe and functional foods.

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