Scientific Papers. Series D. Animal Science. Vol. LXVII, No. 2, 2024
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IN VITRO SCREENING OF LACTIC ACID BACTERIA AS BIOCONTROL AGENTS FOR BIOPRESERVATION OF PERISHABLE AGRO-FOOD PRODUCTS

Florentina BADEA[1], Mircea Cosmin PRISTAVU[1], Constantin Alexandru ALDEA[1], Florentina ISRAEL-ROMING[1], Florentina MATEI[1, 2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania
[2]Transilvania University of Brașov, Faculty of Food Industry and Tourism,
148 Castelului Street, 500014, Brașov, Romania

Corresponding author email: cosmin_mircea96@yahoo.com

Abstract

The aim of this study was to evaluate in vitro the potential of different strains of lactic acid bacteria (LAB), belonging to Enterococcus faecium, Lactobacillus brevis, Lactobacillus farciminis, Lactobacillus fermentum, Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus and Weissella cibaria, for their potential use for increasing the shelf life of different perishable food products. Several screening tests were taken into account, mainly related to their probiotic potential, but also the potential use as biocontrol agents during storage. The strains of P. pentosaceus, L. fermentum, and L. plantarum had the greatest biopreservation potential, with a spectrum of antimicrobial activity against a wide range of pathogenic bacteria and food spoilage fungi. They are capable of producing bacteriocins, exopolysaccharides, organic acids (lactic and acetic acids), enzymes (protease and phytase). In addition, freeze-drying with glucose as a cryoprotective agent resulted in a high survival rate of LAB strains, with a survival rate exceeding 50%. The aforementioned findings suggest that out of the LAB strains tested, L. plantarum MI207 represents a viable option for extending the shelf life of fresh, minimally processed food products in a sustainable manner.

Key words: *lactic acid bacteria, preservation, perishable food, probiotic.*

INTRODUCTION

In view of the demands of modern living and the resulting pressure on one's time, numerous studies have demonstrated that a daily diet rich processed and ultra-processed foods ready-to-eat, characterised by a high calories content and a low nutrients level, can lead to overeating and weight gain (Valicente et al., 2023; Barbaresko et al., 2024; Dicken et al., 2024). Over time, this may give rise to a range of health issues, such as obesity, type 2 diabetes, high blood pressure, heart disease, certain types of cancer, as well as a negative impact on mental health (Juul et al., 2022; Mazloomi et al., 2023; Valicente et al., 2023; Dicken et al., 2024). It is therefore strongly recommended that a diet comprising fresh and minimally processed foods, including vegetables, fruit, and whole grains, be adopted. Such foods provide essential nutrients, including vitamins, minerals, and dietary fibre, which are necessary for a healthy lifestyle. The question thus arises as to how these fresh or minimally processed foods can be kept safe for a longer period without the use of chemical preservatives?

The risk of pathogens' contamination can be reduced when fresh vegetables are processed using appropriate sanitation techniques. To remove soil, grime, debris, and potentially microorganisms from the surface of fresh products, decontamination techniques often involve washing the product with water or a solution of water and disinfectants (Machado et al., 2017; Huang et al., 2018; Yoon et al., 2018). However, these methods may not be sufficient to completely remove biofilm from fruit and vegetable surfaces.

The term "bioprotectant agents" is used in the context of food biocontrol and refers to live microorganisms and/or their metabolites that have been intentionally incorporated into food to inhibit the growth of undesirable microorganisms while preserving the sensory properties of the food. Lactic acid bacteria (LAB) are a group of non-pathogenic

microorganisms (generally recognized as safe, or GRAS) (Axelsson, 2004; Shi et al., 2022). They have a long history of use as starter cultures and probiotics (Patarata et al., 2024). Their multifunctional properties have been exploited to develop a range of functional foods with high-value nutraceuticals and to promote health. LAB can convert carbohydrates into organic acids (mainly lactic acid) and produce a range of metabolites (e.g exoplisaccharides) that enhance the sensory and nutritional qualities of food, as well as antimicrobial components (i.e. bacteriocins) that prolong the shelf life of food products (Varsha et al., 2016; Agriopoulou et al., 2020; Peng et al., 2020; Bhattacharya et al., 2022; Zapaśnik et al., 2022).

While there has been a notable increase in the number of scientific papers published on the subject of utilising LAB species or their metabolites as biocontrol agents in fruit and vegetable preservation, there has not yet emerged a standardised methodology for their implementation (Corbo et al., 2015; Linares-Morales et al., 2018; Agriopoulou et al., 2020; Badea et al., 2022; Sri et al., 2023; Ramos et al., 2024).

The efficacy of LAB as biocontrol agents is affected by a multitude of factors intrinsic to the LAB agents themselves (e.g., specie/s and strain/s) and extrinsic factors (e.g., percent of inoculation (CFU/ml or g), the presence of associated pathogenic bacteria in microbial consortia, the presence of microbial biofilm, physical and chemical properties of the products; maintaining LAB viability during storage conditions, etc.).

Investigation of LAB isolated from indigenous foods and beverages and more could lead to the identification of previously unknown species or strains that may exhibit properties of biotechnological interest (Diguță et al., 2020; Daba et al., 2021, 2022; Pristavu et al., 2022; Ouili et al., 2023; Kouadio et al., 2024).

The genera *Bifidobacterium, Lactococcus, Lactobacillus, Leuconostoc, Pediococcus, Streptococcus,* and *Weisella* are well-known and extensively studied (Daba & Elkhateeb, 2020). LABs, either through the inoculation of viable cells and/or cell-free supernatants or through the incorporation of edible coatings, have been exploited to improve the shelf-life of fresh fruit and vegetables during storage while maintaining sensory properties (Iseppi et al., 2022).

This study targeted the *in vitro* evaluation of several LAB strains for their potential use to increase the shelf-life of different perishable food products and further development of edible coatings for different fresh fruits.

MATHERIALS AND METHODS

1. Lactic acid bacteria strains

A total of 15 LAB strains were used in this study (Table 1). All LAB strains were maintained at -20°C in MRS broth (Man, Rogosa and Sharpe, Oxoid, Limited, Hampshire, United Kingdom) containing 20% glycerol. For the pre-inoculum LABs were cultivated in MRS broth at 37°C for 24 hours.

Table 1. LAB strains used in this study

Species name	Code	Microbial collection
Enterococcus faecium	MI201	UASVM Bucharest
Lactobacillus brevis	MI202	UASVM Bucharest
Lactobacillus farciminis	MI203	UASVM Bucharest
Lactobacillus fermentum	MI204	UASVM Bucharest
Pediococcus pentosaceus	MI205	UASVM Bucharest
Weissella cibaria	MI206	UASVM Bucharest
Lactobacillus plantarum	MI207	UASVM Bucharest
Lactobacillus plantarum	MI208	UASVM Bucharest
Pediococcus acidilactici	MI209	UASVM Bucharest
Pediococcus pentosaceus	MI210	UASVM Bucharest
Pediococcus pentosaceus	MI211	UASVM Bucharest
Lactobacillus brevis	MI212	UASVM Bucharest
Pediococcus pentosaceus	MI213	UASVM Bucharest
Pediococcus pentosaceus	MI214	UASVM Bucharest
Pediococcus acidilactici	MI215	UASVM Bucharest

2. Antimicrobial activity

2.1. Antibacterial activity

Antibacterial activity was assessed against different representative pathogenic bacteria (*Escherichia coli* ATCC 25922, *Listeria ivanovii* ATCC 1911, *Listeria monocytogenes* ATCC 7644, and *Salmonella enterica* serovar Typhimurium ATCC 14028) using two distinct tests such as the dual culture plate assay of the LAB cells and the agar well diffusion assay of the CFSs (cell-free supernatants), as previously described by Balouiri et al. (2016) and Diguță et al. (2020) with minor modifications. Briefly, the reference bacteria were cultivated in trypticase soya broth (TSB, Alliance Bio Expertise, France) at 30°C, for 24 h. An aliquot (10 µL) of an overnight LAB culture was inoculated in a spot on MRS agar distributed on a Petri dish. The reference pathogenic strain was inoculated in Tryptic Soy Agar (TSA, Alliance Bio Expertise, France) maintained at 45°C, and spread over the initially inoculated MRS agar medium. The free-cell supernatant (CFS) was obtained from an overnight LAB culture via centrifugation (at 10,000 x g for 5 min, at 4°C) and filtration through sterile Millipore 0.22 µm filters (Sartorius, Goettingen, Germany). Wells with a diameter of 6 mm were punched aseptically with a sterile tip and filled with 100 µl of the tested CFS. Then, agar plates were incubated at 37°C for 24 hours. The inhibition zone, defined as the area surrounding each well and exhibiting an inhibition size of at least 1 mm, was indicative of the antibacterial efficacy of the LAB cells and the CFS.

2.2. Antifungal activity

The ability of LAB strains to inhibit fungal growth was evaluated against 10 pathogenic fungi, respectively *Aspergillus brasiliensis* ATCC 16404, *A. carbonarius* MI 15, *A. flavus* MI 24, *A. niger* MI 5, *A. ochraceus* MI 2, *Botrytis cinerea* MI Aligote Huși, *Fusarium oxysporum* MI 3, *F. proliferatum* MI 4, *Penicillium digitatum* MI 22, and *P. expansum* MI BB Husi. The antifungal activity was evaluated using a dual culture assay, as previously described by Diguță et al. (2020). An aliquot (10 µL) of an overnight LAB culture was inoculated in a spot on MRS agar distributed on a Petri dish and incubated for 24 h at 30°C. A fresh layer of potato dextrose agar (PDA, Alliance Bio Expertise, France), including a fresh fungal spore suspension (10^6 spores/ml), was then placed over the initially inoculated MRS agar medium. After another 48 h of incubation at 30°C, the size of the zone of inhibition that develops in the immediate proximity of the LAB strains was measured with a ruler.

3. On-plate testing of bacteriocin producing

The bacteriocin production of the LAB strains was determined using the method proposed by Matei et al. (2018). The LAB strains were grown in MRS broth for 24 hours at 37°C and then centrifuged at 5000 rpm for 10 minutes. The supernatant was subsequently collected and neutralised with a 40% sodium hydroxide solution. A volume of 3 µL of the supernatant was added to the MRS agar plate in a spot.

The bacteriocin-sensitive *Streptococcus thermophilus* ATCC 19258 strain was inoculated onto 20 mL of MRS agar maintained at 45°C and then poured into an initially prepared Petri dish. After 24 hours of incubation at 37°C, the appearance of an inhibition zone surrounding the spot indicated a positive result.

4. Exopolysaccharides production assay

LAB strains were inoculated onto MRS broth (containing 5% glucose) and then incubated at 30°C for 48 hours. The methodology used was described by Paulo et al. (2012), with some modifications. To highlight the production of the exopolysaccharides (EPS), the overnight LAB cultures were inoculated on MRS agar supplemented with 30% sucrose, as a carbon source.

After incubation at 30°C for 72 hours, the production of EPS was assessed visually by the formation of mucoid and translucent colonies on the agar plates. The slimy aspect was determined by gently touching them with a sterile inoculation loop.

5. On-plate screening of organic acids production

The production of lactic acid was tested following the methodology described by Kumar et al. (2020). The medium consisted of molasses (150 g/l), malt extract (2.5 g/l), yeast extract (2.5 g/l), peptone (2.5 g/l), and agar (15

g/l), which was distributed in Petri dishes. To enhance lactic acid production, calcium carbonate was incorporated as a neutralizing agent. LAB strains were inoculated in spots (10 µL) and then incubated at 30°C for 2-3 days. Lactic acid production was indicated by the formation of a clear inhibition zone surrounding the LAB colonies.

For the acetic acid, a volume of 10 µL of an active LAB culture was spotted on Chalk agar plates (comprising glucose (10 g/L), yeast extract (3 g/L), calcium carbonate (3 g/L), with agar (20 g/L) as the substrate), according to the methodology described by Sidari et al. (2021), with a few modifications. These were subsequently incubated at 30°C for five days. The presence and degree of a transparent halo surrounding the LAB strains provided evidence of the rate at which acetic acid was produced.

6. Detection and quantification of organic acids content by HPLC

The quantification of organic acids (lactic and acetic acids) was conducted via high-performance liquid chromatography (HPLC), with samples prepared by centrifugation and stored at -18°C. For subsequent analysis, samples were diluted with ultrapure water and then filtered through a 0.20 µM Millex PTFE membrane.

The chromatographic analysis was conducted using a Waters Alliance system, which includes a separation module and a UV detector (both manufactured by Waters; Millipore, Milford, MA, USA). The separation was conducted using two SUPELCOGEL H Guard Columns (250 mm x 4.6 mm and 50 mm x 4.6 mm) with 0.1% H_3PO_4 as the mobile phase. UV detection was performed at 210 nm. All data were analysed using the Empower 2.3 system (Waters Corporation, Milford, MA, USA). The quantification process was based on the peak area, with a calibration curve obtained by injecting different volumes of organic acid standard solutions.

7. On-plate screening of hydrolytic enzymes production

LAB isolates were inoculated in spots on the surface of culture media that had been supplemented with casein (for protease) and sodium phytate (0.2% w/v) (for phytase). The proteolytic activity was determined using culture media containing skim milk (PanReac Applichem, Darmstadt, Germany) in a 1:2 ratio with water (v/v) and 2% agar, as previously described by Sidari et al. (2021). Phytase activity was measured using a methodology previously described by Bhagat et al. (2020). The medium is composed of glucose (0.5%), peptone (1%), yeast extract (0.5%), magnesium sulfate (0.1%), calcium chloride (0.1%), and sodium phytate (0.2%) (Sigma-Aldrich, Missouri, USA). The plates thus prepared were incubated at 30°C for 96 hours. The presence of a translucent halo surrounding LAB strains that exhibited protease and phytase production was considered inductive of the desired outcome.

8. Antioxidant activity

The 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of the tested LAB strains was evaluated using the method previously described by Brand-Williams et al. (1995), with some modifications. Briefly, overnight LAB cultures were centrifuged at 10,000 x g for 5 minutes. 1 ml of a free-cell supernatant was vigorously mixed with 2 ml of 100 µM DPPH solution (dissolved in pure ethanol) (Aldrich, Merck KGaA, Darmstadt, Germany), and then incubated in a dark environment for 30 minutes at room temperature. After this incubation period, the mixture was centrifuged at 10,000 × g for 5 minutes. The absorbance of the supernatant at 517 nm (OD517) was measured utilizing a UV-1800 spectrophotometer (ChromTech, Minneapolis, USA). To ensure the accuracy and reliability of the results, the control samples were substituted with an equivalent volume of distilled water. In the blank sample, DPPH was substituted with an identical volume of absolute ethanol. An equal volume of distilled water and absolute ethanol was employed as a calibration standard, thus enabling the assessment of the accuracy and precision of the results. The percent of radical scavenging activity was estimated using the following equation:

$$AA\ \% = \frac{ABSDPPH - ABSsample}{ABSDPPH} \times 100.$$

where: AA(%) = antioxidant activity (%); ABS_{DPPH} = the absorbance of DPPH solution without any sample; ABS_{sample} = the absorbance of the mixture of 2 mL DPPH and 1 mL sample.

9. Preservation of LAB by freeze-drying procedure

The overnight LAB cells cultivated in MRS broth were harvested by centrifugation at 4000 x g for 10 minutes at 4°C. The cells were washed twice using PBS buffer (VWR Chemicals, Ohio, USA) and suspended in 2 ml of D-glucose (Carl Roth GmbH, Karlsruhe, Germany), which was used as a cryoprotectant agent.

Following freezing overnight at a temperature of -20 °C, the cell suspensions were freeze-dried in a chamber-type freeze-dryer (FreeZone6, LABCONCO, 6 L Benchtop Freeze Dry System, Kansas, MO, USA) at a temperature of -55 °C and an absolute pressure of 0.3 millibars for 4 hours.

Before and following the freeze-drying procedure, the plate count method was employed to assess cell viability. The survival rate of LAB strains was calculated according to the following formula:

$$\text{Viability } \% = \frac{\log \text{CFU/ml after lyophilization}}{\log \text{CFU/ml before lyophilization}} \times 100.$$

10. Statistical analysis

The obtained results are the average of three independent trials conducted under identical conditions. All data are presented as the mean ± standard deviation. The calculations, figures, and boxplots were performed using Excel 2019.

RESULTS AND DISCUSSIONS

1. Antimicrobial activity of LAB strains

All isolates exhibited moderate to high inhibitory activity against *S. enterica* Typhimurium, *L. monocytogenes,* and *L. ivanovii,* as detailed in Table 2. The majority of LAB cells displayed a comparable degree of inhibitory activity against *E. coli,* although the inhibitory effect appeared to be less pronounced in *L. brevis* MI202 and *P. acidilactici* MI215.

Overall, the antibacterial activity of CFSs was significantly lower than that of LAB cells (Table 2). However, the CFSs derived from *Ent. faecium* MI201, *L. brevis* MI202, and *W. cibaria* MI206 strains demonstrated moderate inhibitory effects against both *L. ivanovii* and *L. monocytogenes*. Moreover, the CFSs derived from the *P. pentosaceus* MI205 strain exhibited a strong inhibitory effect on *L. ivanovii* and *L. monocytogenes*.

In a study conducted by Trias et al. (2008), five LAB strains were identified as capable of inhibiting the proliferation of *L. monocytogenes* and *S*. Typhimurium in cut iceberg lettuce leaf. However, they did not demonstrate the same inhibitory effect against *E. coli*. Siroli et al. (2014) demonstrated that a nisin-producing strain of *Leuconostoc lactis* could inhibit the total mesophilic species, *E. coli*, and *L. monocytogenes,* when added at a level of 7 log CFU/ml in the washing solution of minimally-processed lamb's lettuce. *Pediococcus* strains isolated from the Komubucha consortium exhibited high antibacterial activity against *Bacillus cereus, L. ivanovii, L. monocytogenes, Proteus hauseri, S. enterica* Typhimurium, and methicillin-resistant *Staphylococcus aureus*, while exhibited low activity against *E. coli* (Diguță et al., 2020). A further study demonstrated the pronounced inhibitory effects of *Lactiplantibacillus plantarum* strains against *L. monocytogenes, S. enterica* serovar Typhimurium, and *S. aureus*, while their impact on *E. coli* was observed to be moderate (Kouadio et al., 2024). A cocktail of *L. plantarum* and *P. pentosaceus* significantly reduced *L. monocytogenes* and *Salmonella enterica* populations on artificially contaminated fresh strawberries for 7 days (Yin et al., 2022).

Filamentous fungi, particularly species from the genera *Aspergillus, Botrytis, Fusarium,* and *Penicillium*, are responsible for the contamination of fresh fruits and vegetables, resulting in significant economic losses.

Extensive research has demonstrated the inhibitory effect of LAB on the development of the filamentous fungi, due to the effect of their biosynthesized metabolites which disrupt the integrity of the fungal cell membrane and inhibit the uptake of amino acids by the fungus (Marie et al., 2018; Sadiq et al., 2019; Nasrollahzadeh et al., 20221; Shi et al., 2022).

In our study the lactic acid strains showed low antifungal activity against *Aspergilli* group. Only strains *L. brevis* MI212, *P. pentosaceus* MI211, *Ent. faecium* MI201, *L. farciminis* MI203 and *L. fermentum* MI204 showed

inhibitory activity against *A carbonarius*; *L. brevis* MI202 and *L. fermentum* MI204 against *A. brasiliensis*, respectively. The highest inhibitory activity was shown by *L. brevis* MI212 against *A. carbonarius*. No other LAB strain demonstrated antifungal activity against *A. flavus, A. niger,* and *A. ochraceus*.

Table 2. Antibacterial activity of the LAB strains

LAB strains	*L. monocytogenes* ATCC 7644		*L. ivanovii* ATCC 19119		*E. coli* ATCC 25922		*S.* Typhimurium ATCC 14028	
	cells	CFS	cells	CFS	cells	CFS	cells	CFS
Ent. faecium MI201	+++	++	+++	++	++	+	+++	+
L. brevis MI202	+++	++	+++	++	+	+	+++	+
L. *farciminis* MI203	+++	+	+++	+	+++	+	+++	+
L. fermentum MI204	+++	+	+++	+	+++	+	+++	+
Pediococcus pentosaceus MI205	++	+++	++	+++	+++	+	+++	+
Weissella cibaria MI206	+++	++	+++	++	+++	+	+++	+
L. plantarum MI207	+++	+	+++	+	+++	+	+++	+
L. plantarum MI208	+++	+	+++	+	+++	+	+++	+
P. acidilactici MI209	++	+	++	+	+++	+	+++	+
P. pentosaceus MI210	+++	+	++	+	+++	+	+++	+
P. pentosaceus MI211	++	+	++	+	+++	+	+++	+
L. brevis MI212	+++	+	++	+	+++	+	+++	+
P. pentosaceus MI213	++	+	++	+	++	+	++	+
P. pentosaceus MI214	+++	+	+++	+	++	+	+++	+
P. acidilactici MI215	+++	+	+++	+	+	+	+++	+

*(-) inhibitory activity absent; (+) inhibition halo of 1-5 mm diameter; (++) inhibition halo of 5-10 mm diameter; (+++) inhibition halo of >10 mm diameter.

Diguță et al. (2020) observed that some *Pediococcus* strains exhibited low antifungal activity against *A. flavus* and *A. niger*. Conversely, five strains of *P. pentosaceus* isolated by Ouili et al. (2023) demonstrated a high ability to suppress the growth of *A. flavus*. The *L. fermentum* MI204, *L. plantarum* MI207, *P. pentosaceus* MI210, and *P. pentosaceus* MI211 strains exhibited pronounced antifungal activity against *B. cinerea*.

Table 3. Antifungal activity of the LAB strains

Bacterii lactice	*A. brasiliensis*	*A. carbonarius*	*A. flavus*	*A. niger*	*A. ochraceus*	*B. cinerea*	*F. oxysporum*	*F. proliferatum*	*P. digitatum*	*P. expansum*
Ent. faecium MI201	-	+	-	-	-	-	-	-	+++	+
L. brevis MI202	+	-	-	-	-	-	+	-	+++	+
L. farciminis MI203	-	+	-	-	-	-	++	+++	+++	+
L. fermentum MI204	+	+	-	-	-	+++	++	+++	+++	+
P. pentosaceus MI205	-	-	-	-	-	-	+	-	-	-
W. cibaria MI206	-	-	-	-	-	-	+	-	-	+
L. plantarum MI207	-	-	-	-	-	+++	+	+++	+	+
L. plantarum MI208	-	-	-	-	-	-	++	+++	++	++
P. acidilactici MI209	-	-	-	-	-	-	-	+	-	-
P. pentosaceus MI210	-	-	-	-	-	+++	++	+	+	+
P. pentosaceus MI211	-	++	-	-	-	++	-	+++	+	-
L. brevis MI212	-	+++	-	-	-	-	+++	+++	+++	+++
P. pentosaceus MI213	-	-	-	-	-	-	-	-	-	+
P. pentosaceus MI214	-	-	-	-	-	-	-	-	-	-
P. acidilactici MI215	-	-	-	-	-	-	-	-	-	-

*(-) inhibitory activity absent; (+) inhibition halo of 1-5 mm diameter; (++) inhibition halo of 5-10 mm diameter; (+++) inhibition halo of >10 mm diameter

A spectrum of activity was observed against *F. oxysporum* and *F. proliferatum*, with the *L. brevis* MI212 strains demonstrating a notable antimicrobial capacity against both *Fusarium* species (Table 3). The effectiveness of LAB strains against *Penicillium* sp. was demonstrated to vary considerably. *Ent. faecium* MI201, *L. brevis* (MI202 and MI212), *L. farciminis* MI203, and *L. fermentum* MI204 demonstrated a notable capacity to inhibit the

growth of *P. digitatum*, while *L. brevis* MI212 exhibited a similar effect on *P. expansum*, as shown in Table 3. The results presented here are consistent with those previously published by Diguță et al. (2020).

Matei et al. (2017) revealed that the Kombucha consortium (or SCOBY which stands for symbiotic acetic/lactic bacteria and yeast) exhibited the most significant inhibition against *B. cinerea*, less significant inhibition against *P. expansum,* and no inhibition against *A. carbonarius* and *A. flavus*.

2. Bacteriocin and EPS production

Lactic acid bacteria are known to produce bacteriocins, which have been demonstrated to possess significant bioactive antimicrobial properties (Daba & Elkhateeb, 2020; Islam et al., 2020; Daba et al., 2022; Bhattacharya et al., 2022; Pristavu et al., 2022; Perez et al., 2022; Kumar et al., 2023). These bacteriocins could potentially be utilised as a natural preservative and may represent a promising alternative to antibiotics, given their efficacy in controlling foodborne pathogens (Daba & Elkhateeb, 2020; Bhattacharya et al., 2022; Perez et al., 2022). The LAB strains that demonstrated the ability to inhibit the growth of the susceptible strain of *S. thermophilus* ATCC 19258 are considered producers of bacteriocins or other active compound(s). A summary of the data on the antimicrobial activity of the crude bacteriocins present in cell-free culture supernatants of LAB strains against *S. thermophilus* ATCC 19258 is presented in Table 4.

The results demonstrated that *L. fermentum* MI204, *L. brevis* MI212, *P. pentosaceus* (MI211, MI213, and MI214) and *P. acidilactici* (MI 209 and MI215) strains exhibited the greatest inhibition zones surrounding the inoculation spots. Additionally, *Ent. faecium* MI201, *L. brevis* MI202, and *P. pentosaceus* MI205 strains can produce some active compounds with inhibitory activity against the tester strain. In a study conducted by Hwanhlem et al. (2014), *Lactococcus lactis* subsp. *lactis* 4KT2W2L and *Ent. faecalis* (KT2W2G, TS9S17, and TS9S19) strains were identified as producers of bacteriocin-like inhibitory substances against some bacteria as indicators, using the agar well diffusion assay. In a relatively published study, Matei et al. (2018) identified three strains of *P. pentosaceus* isolated from the Kombucha consortium as bacteriocin producers.

Moreover, EPS produced by certain genera of LAB have attracted considerable interest due to their ability to extend the shelf life of products, enhance the functional properties of dairy and food products, and promote health (Daba et al., 2021; Korcz, & Varga, 2021; Jurášková et al., 2022).

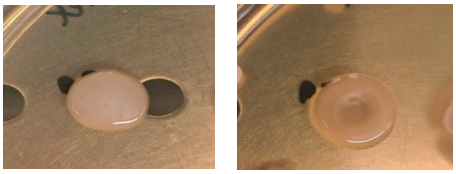

Figure 1. Appearance of colonies producing exopolysaccharides (own source)

Figure 1 illustrates the characteristics of the EPS-producing LAB strains, which were observed to be white, mucoid, and translucent colonies on MRS agar supplemented with 30% sucrose.

Table 4. Bacteriocin and EPS production by LAB strains

LAB strains	Inhibition zone*	EPS production**
Ent. faecium MI201	+	-
L. brevis MI202	+	-
L. farciminis MI203	-	-
L. fermentum MI204	++	+
P. pentosaceus MI205	+	+
W. cibaria MI206	-	-
L. plantarum MI207	-	+
L. plantarum MI208	-	-
P. acidilactici MI209	++	-
P. pentosaceus MI210	-	-
P. pentosaceus MI211	+++	-
L. brevis MI212	++	-
P. pentosaceus MI213	+++	-
P. pentosaceus MI214	+++	-
P. acidilactici MI215	+++	-

*(-) inhibitory activity absent; (+) inhibition halo of 1-5 mm diameter; (++) inhibition halo of 5-10 mm diameter; (+++) inhibition halo of >10 mm diameter

**(-) absent activity; (+) present activity

Screening results revealed that the strains *L. fermentum* MI204, *P. pentosaceus* MI205, and *L. plantarum* MI207 exhibited the potential to produce exopolysaccharides (Table 4). In another study, Ma'unatin et al. (2020) demonstrated that *Fructobacillus fructosus* N4 and *Leuconostoc mesenteroides* (N5, N7, N9, N10) strains were capable of producing

extracellular polymeric substances (EPS) when cultivated on MRS agar with a 30% sucrose supplement. Álvarez et al. (2021) showed how a *Lactobacillus plantarum* strain integrated in an edible EPS-based coating from *Weissella confusa* improved the microbiological and physicochemical quality of cherry tomatoes. According to reports, a significant component of the biofilms' resistance mechanism is the EPS matrix.

3. Organic acids production

The available literature suggests that the preservation of LAB is the consequence not of the action of a single compound but rather of the combined effect of metabolites produced by these microorganisms during fermentation.

In the present study, lactic and acetic acids in the CFS of LAB strains were ascertained through using two distinct methods such as plate screening on specific media and HPLC assay. The results from these investigations are presented in Tables 5 and 6, respectively. LAB strains demonstrated the ability to produce organic acids during fermentation. The formation of the largest halos around the inoculation area indicated the highest potential of these strains for lactic acid production. With the exception of *L. fermentum* MI207 and *P. pentosaceus* MI214, the remaining LAB strains were observed to produce acetic acid on solid medium. Of these, *Ent. faecium* MI201, *L. brevis* MI202, and *L. farciminis* MI203 exhibited the highest activity (Table 5).

Table 5. Detection of acetic and lactic acids production of LAB strains through plate screening

LAB strains	Acetic acid production	Lactic acid production
Ent. faecium MI201	+++	++
L. brevis MI202	+++	++
L. farciminis MI203	+++	+
L. fermentum MI204	++	+++
P. pentosaceus MI205	++	+
W. cibaria MI206	++	+
L. plantarum MI207	-	+++
L. plantarum MI208	++	++
P. acidilactici MI209	++	+++
P. pentosaceus MI210	++	++
P. pentosaceus MI211	++	+
L. brevis MI212	++	++
P. pentosaceus MI213	++	++
P. pentosaceus MI214	-	++
P. acidilactici MI215	++	+

*(-) halo absent; (+) halo of 1-5 mm diameter; (++) halo of 5-10 mm diameter; (+++) halo of >10 mm diameter

On the other hand, all LAB strains were found to be able to produce lactic acid on agar medium (Table 5), with *L. fermentum* MI204 and MI207, and *P. acidilactici* MI209 strains demonstrating the greatest ability.

Table 6. Quantification of lactic and acetic acids content by HPLC assay

LAB strains	Acetic acid mg/ml	Lactic acid mg/ml
Ent. faecium MI201	0.42	14.17
L. brevis MI202	0.53	9.43
L. farciminis MI203	0.92	6.64
L. fermentum MI204	**1.55**	16.43
P. pentosaceus MI205	<LoQ	6.46
W. cibaria MI206	0.80	10.96
L. plantarum MI207	0.60	7.04
L. plantarum MI208	0.22	12.47
P. acidilactici MI209	**1.30**	16.86
P. pentosaceus MI210	0.71	14.93
P. pentosaceus MI211	1.05	**20.02**
L. brevis MI212	0.50	15.17
P. pentosaceus MI213	0.47	**20.41**
P. pentosaceus MI214	<LoD	16.06
P. acidilactici MI215	0.69	**27.00**

<LoQ - limit of quantification; <LoD - detection limit

The quantified level of acetic acid by HPLC exhibits considerably lower values, ranging from 0.22 mg/ml for *L. plantarum* MI208 to 1.55 mg/ml for *L. fermentum* MI204. The concentration of acetic acid was below the limit of detection for *P. pentosaceus* MI214 and below the limit of quantification for *P. pentosaceus* MI205 (Table 6). The lactic acid concentration, the principal organic acid with beneficial effects, exhibited a considerable range from 6.46 mg/ml for *P. pentosaceus* MI205 to 27 mg/ml for *P. acidilactici* MI215 (Table 6).

According to Loubiere et al. (1997), lactic acid exerts its inhibitory effects on metabolic processes and cellular proliferation, possibly due to the synergistic interaction between these compound and other secondary metabolites, including acetic and formic acids.

4. Proteolytic and phytase activities

The potential for LAB strains to produce proteases was evaluated on agar skim milk medium. Of the strains tested, nine demonstrated positive proteolytic activity, with the greatest halo surrounding the *Ent. faecium* MI201, *P. acidilactici* MI209, *L. brevis* MI212, and *P. acidilactici* MI215 strains (Table 7).

These outcomes align with those of previous studies in this area. *L. plantarum* subsp. *plantarum* P15 and *E. faecalis* ZZUPF95 exhibited the greatest protease production abilities, as previously reported by Ma et al. (2022). Additionally, Kouadio et al. (2024) reported the production of protease enzymes by ten *L. plantarum* strains, isolated from traditional fermented dockounou paste.

Table 7. Proteolytic and phytase activity of the LAB strains

LAB strains	Proteolytic activity	Phytase activity
Ent. faecium MI201	+++	+++
L. brevis MI202	++	+++
L. farciminis MI203	-	+++
L. fermentum MI204	-	+++
P. pentosaceus MI205	++	+++
W. cibaria MI206	-	+++
L. plantarum MI207	-	+++
L. plantarum MI208	+	+++
P. acidilactici MI209	+++	+++
P. pentosaceus MI210	++	+++
P. pentosaceus MI211	-	+++
L. brevis MI212	+++	-
P. pentosaceus MI213	-	+++
P. pentosaceus MI214	++	+++
P. acidilactici MI215	+++	+++

*(-) halo absent; (+) halo of 1-5 mm diameter; (++) halo of 5-10 mm diameter; (+++) halo of >10 mm diameter

Previous studies demonstrated that different LAB strains possess the ability to produce intracellular and/or extracellular phytases (Cizeikiene et al., 2015; Bhagat et al., 2020; Sharma et al., 2020).

In the present study, all LAB strains presented extracellular phytase activities that were significantly elevated after 96 hours of incubation, with the sole exception of *L. brevis* MI212.

As reported by Cizeikiene et al. (2015), *P. acidilactici* (KTU05-7) and *P. pentosaceus* KTU05-8 have been identified as the strains with the highest extracellular phytase activity. Also, another strain of *P. acidilactici* SMVDUDB2, isolated from traditional fermented cheese product, has been demonstrated to exhibit extracellular phytase enzyme activity, with an optimum pH of 5.5 and temperature of 37°C, being thermostable at 60°C (Bhagat et al., 2020). Moreover, *L. brevis* strain can synthesise phytase both within and outside their cells (Sümengen et al., 2012).

5. Antioxidant activity

The antioxidant potential of LAB strains was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The findings indicated that all fifteen LAB strains demonstrated notable antioxidant activity ranging from 45.79% to 52.58% (Figure 2). Among the strains tested, *P. pentosaceus* MI205 displayed the most pronounced antioxidant efficacy, exhibiting a DPPH activity of 52.58%.

These findings are consistent with those previously reported in the literature (Diguță et al., 2020; Koaudio et al., 2024).

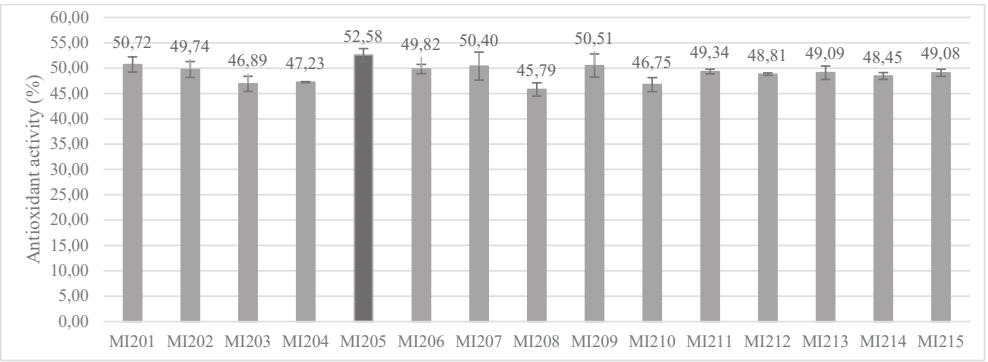

Figure 2. Antioxidant activity (%) of LAB strains

6. Preservation of LAB strains by lyophilization

The freeze-drying (or lyophylization) procedure is frequently employed to preserve and store LAB strains for biotechnological applications, with the advantage of maintaining the native microbial structure and viability of bacterial cells during storage (Meena et al., 2023). However, the process of freezing and subsequent freeze-drying leads to membrane damage and osmolarity stress due to the formation of ice crystals during the freezing step. Thus cryoprotective agents are required to extend the viability of LAB bacteria.

In our study, glucose was employed as a cryoprotective agent to enhance the viability of microorganisms during the freeze-drying procedure and facilitate their future utilization. The viability percentage of freeze-dried LAB strains ranged from 50.68% (*Ent. faecium* MI201) to 98.40% (*L. plantarum* MI207) (Figure 3). There is considerable variability in the efficacy of glucose as a cryoprotectant agent between different LAB strains.

In their study, Diguță et al. (2020) investigated the storage stability of the probiotic *Pediococcus* sp. through freeze-drying techniques utilising two different cryoprotectants: glucose and sucrose. Their findings indicated a high viability rate of 86–92%, which provides evidence of the effectiveness of their methodology. Consequently, further research is required to select the most appropriate cryoprotectant agents for conditioning LAB strains, with the highest viability rates and cost-effectiveness.

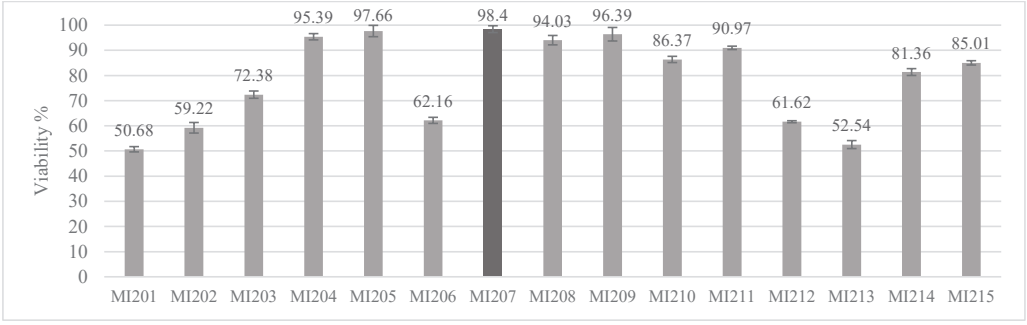

Figure 3. Viability after lyophilization (%) of LAB strains

CONCLUSIONS

The challenge of maintaining the nutritional value and sensory properties of perishable fruits for a longer period while ensuring its suitability for distribution and consumption is significant in terms of technological obstacles. Among the fifteen LAB strains tested, *Lactobacillus plantarum* MI207 demonstrated the greatest efficacy against foodborne pathogens, as well as several other beneficial abilities due to their probiotic properties. In addition, the EPS production is encouraging for testing the positive LABs for the fruit coating potential. Nevertheless, there is still much to be researched regarding the potential of *Lactobacillus plantarum* MI207 as a sustainable candidate for enhancing the shelf-life of fresh and minimally processed foods.

REFERENCES

Agriopoulou, S., Stamatelopoulou, E., Sachadyn-Król, M., & Varzakas, T. (2020). Lactic acid bacteria as antibacterial agents to extend the shelf life of fresh and minimally processed fruits and vegetables: Quality and safety aspects. *Microorganisms*, *8*(6), 952.

Álvarez, A., Manjarres, J. J., Ramírez, C., & Bolívar, G. (2021). Use of an exopolysaccharide-based edible coating and lactic acid bacteria with antifungal activity to preserve the postharvest quality of cherry tomato. *Lwt*, *151*, 112225.

Axelsson, L. (2004). Lactic acid bacteria: classification and physiology. Food Science and Technology-New York-Marcel Dekker-, 139, 1-66.

Badea, F., Diguță, C. F., & Matei, F. (2022). The use of lactic acid bacteria and their metabolites to improve the shelf life of perishable fruits and vegetables. *Sci. Bull. Series F. Biotechnologies*, *26*(1), 117-125.

Balouiri, M., Sadiki, M., Ibnsouda, S.K. (2016). Methods for *in vitro* evaluating antimicrobial activity: a review. *Journal of Pharmaceutical Analysis*, 6(2):71–9.

Barbaresko, J., Bröder, J., Conrad, J., Szczerba, E., Lang, A., & Schlesinger, S. (2024). Ultra-processed food consumption and human health: an umbrella review of systematic reviews with meta-analyses. *Critical Reviews in Food Science and Nutrition*, 1-9.

Bhagat, D., Raina, N., Kumar, A., Katoch, M., Khajuria, Y., Slathia, P. S., & Sharma, P. (2020). Probiotic properties of a phytase producing *Pediococcus acidilactici* strain SMVDUDB2 isolated from traditional fermented cheese product, Kalarei. *Scientific Reports*, *10*(1), 1926.

Bhattacharya, D., Nanda, P. K., Pateiro, M., Lorenzo, J. M., Dhar, P., & Das, A. K. (2022). Lactic acid bacteria and bacteriocins: novel biotechnological approach for biopreservation of meat and meat products. *Microorganisms*, *10*(10), 2058.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, *28*(1), 25-30.

Cizeikiene, D., Juodeikiene, G., Bartkiene, E., Damasius, J., & Paskevicius, A. (2015). Phytase activity of lactic acid bacteria and their impact on the solubility of minerals from wholemeal wheat bread. *International Journal of Food Sciences and Nutrition*, *66*(7), 736-742.

Corbo, M. R., Campaniello, D., Speranza, B., Bevilacqua, A., & Sinigaglia, M. (2015). Non-conventional tools to preserve and prolong the quality of minimally-processed fruits and vegetables. *Coatings*, *5*(4), 931-961.

Daba, G. M., & Elkhateeb, W. A. (2020). Bacteriocins of lactic acid bacteria as biotechnological tools in food and pharmaceuticals: Current applications and future prospects. *Biocatalysis and Agricultural Biotechnology*, *28*, 101750.

Daba, G. M., Elnahas, M. O., & Elkhateeb, W. A. (2021). Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical, and medical applications. *International Journal of Biological Macromolecules*, *173*, 79-89.

Daba, G. M., Elnahas, M. O., & Elkhateeb, W. A. (2022). Beyond biopreservatives, bacteriocins biotechnological applications: History, current status, and promising potentials. *Biocatalysis and Agricultural Biotechnology*, *39*, 102248.

Dicken, S. J., & Batterham, R. L. (2024). Ultra-processed Food and Obesity: What Is the Evidence? *Current Nutrition Reports*, 1-16.

Diguță, C. F., Nițoi, G. D., Matei, F., Luță, G., & Cornea, C. P. (2020). The biotechnological potential of *Pediococcus* spp. isolated from Kombucha microbial consortium. *Foods*, *9*(12), 1780.

Huang, K., Wrenn, S., Tikekar, R., & Nitin, N. (2018). Efficacy of decontamination and a reduced risk of cross-contamination during ultrasound-assisted washing of fresh produce. *Journal of Food Engineering*, *224*, 95-104.

Hwanhlem, N., Chobert, J. M., & Aran, H. (2014). Bacteriocin-producing lactic acid bacteria isolated from mangrove forests in southern Thailand as potential bio-control agents in food: Isolation, screening and optimization. *Food Control*, *41*, 202-211.

Islam, R., Hossain, M. N., Alam, M. K., Uddin, M. E., Rony, M. H., Imran, M. A. S., & Alam, M. F. (2020). Antibacterial activity of lactic acid bacteria and extraction of bacteriocin protein. *Advances in Bioscience and Biotechnology*, *11*(2), 49-59.

Iseppi, R., Zurlini, C., Cigognini, I. M., Cannavacciuolo, M., Sabia, C., & Messi, P. (2022). Eco-friendly edible packaging systems based on live-*Lactobacillus* kefiri MM5 for the control of *Listeria monocytogenes* in fresh vegetables. *Foods*, *11*(17), 2632.

Jurášková, D., Ribeiro, S. C., & Silva, C. C. (2022). Exopolysaccharides produced by lactic acid bacteria: from biosynthesis to health-promoting properties. *Foods*, *11*(2), 156.

Juul, F., Deierlein, A. L., Vaidean, G., Quatromoni, P. A., & Parekh, N. (2022). Ultra-processed foods and cardiometabolic health outcomes: from evidence to practice. *Current Atherosclerosis Reports*, *24*(11), 849-860.

Korcz, E., & Varga, L. (2021). Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. *Trends in Food Science & Technology*, *110*, 375-384.

Kouadio, N. J., Zady, A. L. O., Kra, K. A. S., Diguță, F. C., Niamke, S., & Matei, F. (2024). *In vitro* probiotic characterization of *Lactiplantibacillus plantarum* strains isolated from traditional fermented dockounou paste. *Fermentation*, *10*(5), 264.

Kumar, G., & Sharma, B. B. Biotechnical Production of Lactic Acid: Effect of Orotic Acid as an Efficient Promoting Agent. *IOSR Journal of Applied Chemistry, 13*(12),10-14.

Kumar, A., Ruhal, R., & Kataria, R. (2023). Bacteriocins of Lactic Acid Bacteria as a Potential Antimicrobial Peptide. *Biomimicry Materials and Applications*, 83-103.

Linares-Morales, J. R., Gutiérrez-Méndez, N., Rivera-Chavira, B. E., Pérez-Vega, S. B., & Nevárez-Moorillón, G. V. (2018). Biocontrol processes in fruits and fresh produce, the use of lactic acid bacteria as a sustainable option. *Frontiers in Sustainable Food Systems*, *2*, 50.

Loubiere, P., Cocaign-Bousquet, M., Matos, J., Goma, G., & Lindley, N. D. (1997). Influence of end-products inhibition and nutrient limitations on the growth of *Lactococcus lactis* subsp. *lactis*. *Journal of Applied Microbiology*, *82*(1), 95-100.

Ma, H., Wang, L., Yu, H., Wang, W., Wu, G., Qin, G., Tan, Z., Wang, Y., & Pang, H. (2022). Protease-producing lactic acid bacteria with antibacterial properties and their potential use in soybean meal fermentation. *Chemical and Biological Technologies in Agriculture*, *9*(1), 40.

Machado, M. C. D. M. M., de Lyra, G. R., da Silva, E. M. M., & de SAo JosE, J. F. B. (2017). Sanitization protocols applied to commercial restaurants: Effects on natural contaminant microbiota and *Salmonella enterica* Enteritidis adhered on tomatoes. *African Journal of Microbiology Research*, *11*(46), 1649-1656.

Marie, K. P., Ngoufack François, Z., Edith Marius, F. K., Ciobotaru, O., Matei, F., Cornea, C. P., & Israel-Roming, F. (2018). Antifungal activity of lactic acid bacteria isolated from peanuts, gari, and orange fruit juice against food aflatoxigenic molds. *Food Biotechnology*, *32*(4), 237-256.

Matei, B., Matei, F., Diguță, C., & Popa, O. (2017). Potential use of Kombucha crude extract in postharvest grape moulds control. *Scientific Bulletin Series F. Biotehnologies,* XXI, 77-80

Matei, B., Salzat, J., Diguță, F. C., Cornea, P. C., Luță, G., Uțoiu E.R.,. Matei, F. (2018). Lactic acid bacteria strains isolated from Kombucha with potential probiotic effect. *Romanian Biotechnological Letters*, *23*(3), 13592-13598.

Ma'unatin, A., Harijono, H., Zubaidah, E., Rifa'i M. (2020). The isolation of exopolysaccharide-producing lactic acid bacteria from lontar (*Borassus flabellifer* L.) sap. *Iran J. of Micro., 12*(5), 437-444.

Mazloomi, S. N., & al. (2023). The association of ultra-processed food consumption with adult mental health disorders: a systematic review and dose-response meta-analysis of 260,385 participants. *Nutritional Neuroscience*, *26*(10), 913-931.

Meena, K. K., Taneja, N. K., Ojha, A., & Meena, S. (2023). Application of spray-drying and freeze-drying for microencapsulation of lactic acid bacteria: A review. *Annals of Phytomedicine*, *12*(1), 706-716.

Nasrollahzadeh, A., Mokhtari, S., Khomeiri, M., & Saris, P. E. (2022). Antifungal preservation of food by lactic acid bacteria. *Foods*, *11*(3), 395.

Ouili, A. S., Diguță, C. F., Maiga, Y., Compaore, C. O. T., Ouattara, A. S., Israel-Roming, F., & Matei, F. (2023). Antifungal activity of *Pediococcus pentosaceus* isolated from bambara groundnut (*Vigna subterranea* (L.) Verdc.) seeds against *Aspergillus flavus*. *AgroLife Scientific Journal*, *12*(2), 125-133.

Patarata, L., Munekata, P. E. S., & dos Ramos Fraqueza, M. J. (2024). Probiotics as starter and nonstarter cultures in fermented foods. In *Strategies to Improve the Quality of Foods* (pp. 203-233). Academic Press.

Paulo, E. M. & al.. (2012). An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation. *Food Science and Technology*, *32*, 710-714.

Peng, K., Koubaa, M., Bals, O., & Vorobiev, E. (2020). Recent insights in the impact of emerging technologies on lactic acid bacteria: A review. *Food Research International*, *137*, 109544.

Perez, R. H., Zendo, T., & Sonomoto, K. (2022). Multiple bacteriocin production in lactic acid bacteria. *Journal of Bioscience and Bioengineering*, *134*(4), 277-287.

Pristavu, M. C., Diguță, C., Coulibaly, W. H., Fanche, S. A. Y., Dopcea, G., & Matei, F. (2022). A review of postbiotics as new health promoters. *AgroLife Scientific Journal*, *11*(2), 142-152.

Ramos, B., Brandão, T. R., Teixeira, P., & Silva, C. L. (2024). Biopreservation of Vegetables. *Novel Approaches in Biopreservation for Food and Clinical Purposes*, Garcia-Gutierrez, E., N. Gomez-Torres, & S. Arbulu (Eds.)., (pp. 41-65), CRC Press.

Sadiq, F. A., Yan, B., Tian, F., Zhao, J., Zhang, H., & Chen, W. (2019). Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: a comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), 1403-1436.

Shi, C., & Maktabdar, M. (2022). Lactic acid bacteria as biopreservation against spoilage molds in dairy products–A review. *Frontiers in Microbiology*, *12*, 819684.

Sharma, N., Angural, S., Rana, M., Puri, N., Kondepudi, K. K., & Gupta, N. (2020). Phytase producing lactic acid bacteria: Cell factories for enhancing micronutrient bioavailability of phytate rich foods. *Trends in Food Science & Technology*, *96*, 1-12.

Sidari, R. & al. (2021). Wine yeasts selection: Laboratory characterization and protocol review. *Microorganisms*, *9*(11), 2223.

Siroli, L., Patrignani, F., Salvetti, E., Torriani, S., Gardini, F., Lanciotti, R. (2014). Use of a nisin-producing *Lactococcus lactis* strain, combined with thyme essential oil, to improve the safety and shelf-life of minimally processed lamb's lettuce. *Proceeding of the 11th International Symposium on lactic acid bacteria, Egmond aan Zee*, The Netherlands, 31 August–4 September 2014.

Sri Sneha, J. V., & Dharumadurai, D. (2023). Biopreservation of Fruits and Vegetables Using Postbiotics. In *Postbiotics* (pp. 363-368). New York, USA: Springer US Publishing House.

Sümengen, M., Dincer, S., & Kaya, A. (2012). Phytase production from *Lactobacillus brevis*. *Turkish Journal of Biology*, *36*(5), 533-541.

Trias, R., Badosa, E., Montesinos, E., & Bañeras, L. (2008). Bioprotective *Leuconostoc* strains against *Listeria monocytogene*s in fresh fruits and vegetables. *International Journal of Food Microbiology*, *127*(1-2), 91-98.

Yin, H. B., Chen, C. H., Colorado-Suarez, S., & Patel, J. (2022). Biocontrol of *Listeria monocytogenes* and *Salmonella enterica* on fresh strawberries with lactic acid bacteria during refrigerated storage. *Foodborne Pathogens and Disease*, *19*(5), 324-331.

Yoon, J. H., & Lee, S. Y. (2018). Comparison of the effectiveness of decontaminating strategies for fresh fruits and vegetables and related limitations. *Critical Reviews in Food Science and Nutrition*, *58*(18), 3189-3208.

Valicente, V. M., Peng, C. H., Pacheco, K. N., Lin, L., Kielb, E. I., Dawoodani, E., Abdollahi A, & Mattes, R. D. (2023). Ultra-processed foods and obesity risk: a critical review of reported mechanisms. *Advances in Nutrition*, *14*(4), 718-738.

Varsha, K.K. & Nampoothiri, K.M. (2016). Appraisal of lactic acid bacteria as protective cultures. *Food Control*, *69,* 61–64, 1

Zapaśnik, A., Sokołowska, B., & Bryła, M. (2022). Role of lactic acid bacteria in food preservation and safety. *Foods*, *11*(9), 1283.