

## ANTIMICROBIAL ACTIVITY OF *PEDIOCOCCUS* STRAINS AGAINST SOME *LISTERIA* SPP. DURING CO-CULTIVATION UNDER STATIC GROWTH CONDITIONS

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### ABSTRACT

Pediococci are lactic acid bacteria widely used as starter cultures in the food industry, which produce lactic acid and bacteriocins (pediocins) possessing significant antilisterial potential. This research aimed to investigate the antimicrobial activity of three *Pediococcus* strains (*Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Pediococcus damnosus*) against three *Listeria* strains (*Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii*) to determine the growth characteristics and kinetic parameters during the process of co-cultivation at 37 ± 1°C. The results demonstrated that *P. pentosaceus* possessed a bacteriostatic effect on *L. monocytogenes*, but a bactericidal effect on *L. innocua* and *L. ivanovii*, and on the 60-th h no viable cells of both pathogens were detected. *P. acidilactici* demonstrated a strong bactericidal effect on the three *Listeria* strains, as *L. monocytogenes* was completely inhibited on the 48-th h and *L. innocua* and *L. ivanovii* were inhibited on the 60-th h of co-cultivation. In contrast, *P. damnosus* showed only a bacteriostatic effect on the three pathogens tested. Therefore, the strains *P. pentosaceus* and *P. acidilactici* can be successfully applied in the food industry as biopreservatives and as means to improve the food safety by inhibition of *Listeria* spp., which are among the most common pathogens in food products of animal origin.

**Keywords:** Antimicrobial activity, co-cultivation, *Pediococcus* sp., *Listeria* sp., pediocins

### INTRODUCTION

The genus *Pediococcus* unifies Gram-positive, catalase-negative, oxidase-negative, facultatively aerobic and homofermentative coccus-shaped bacteria, which produce lactic acid as major end product of glucose fermentation (Franz *et al.*, 2014). They are widely used as starter cultures in the fermentation of meat products, vegetables, sourdough bread making (Plessas *et al.*, 2020) and silage production (Cai *et al.*, 1999). Some *Pediococcus* strains have been isolated from dairy products (farm-style cheeses and Cheddar cheese) as a part of non-starter lactic acid bacteria (Gurira & Buys, 2005) or can be considered as spoilage bacteria in beer (Sakamoto & Konings, 2003; Iijima *et al.*, 2007), wine (Beneduce *et al.*, 2004) and other beverages.

The members of genus *Pediococcus* are known to possess many health benefits such as protection against cardiovascular diseases, inhibition of pathogenic microorganisms in the gastrointestinal tract and immunomodulatory effects that make them suitable for use as probiotic strains (Nghe & Nguyen, 2014). One of the most important features of *Pediococcus* spp. is their antimicrobial activity against broad range of foodborne pathogens and spoilage microorganisms. The inhibitory effect of pediococci is related to the production of lactic acid that reduces the pH level in the food matrix during fermentation process and the synthesis of antimicrobial peptides known as bacteriocins or pediocins, which find practical application in food biopreservation. According to the bacteriocin classification, pediocins belong to class IIa bacteriocins characterized as small unmodified peptides (<5 kDa) consisting of 36-48 amino acid residues (Porto *et al.*, 2017). *P. acidilactici* and *P. pentosaceus* are among the main producers of pediocins and their isoforms with possible utilization in the food industry as biopreservatives prolonging the shelf life of different food products (Mora *et al.*, 2003). Many studies revealed that pediocins represent effective antilisterial bacteriocins, which act specifically against the foodborne pathogen *Listeria monocytogenes*. In addition to their high antimicrobial potential, pediocins display some advantages (higher antilisterial activity, wide pH range effectiveness and heat stability) over nisin, a bacteriocin produced by *Lactococcus lactis* subsp. *lactis*, officially approved in many countries as generally recognized as safe (GRAS) and widely used as biopreservative in the food industry, especially in the dairy sector (Jamuna & Jeevaratnam, 2004; Pal *et al.*, 2010). Some studies reported that *Pediococcus* bacteriocins possess greater biopreservation properties in comparison with the conventional chemical preservatives, such as sodium sulphite and sodium benzoate, which can cause various negative effects on human health (Skariyachan & Govindarajan, 2019).

The lactic acid bacteria have always been of paramount importance in the food industry due to their application as starter cultures, probiotics and functional food ingredients, texture, flavour and aroma enhancers, and last, but not at least – as biopreservation agents. In recent years, the use of *Pediococcus* spp. in the food industry attracted the research attention due to their valuable technological properties, which are essential for the final food quality (Adesulu-Dahunsi *et al.*, 2021). The bacteriocin production and high antimicrobial activity of *Pediococcus* spp. against some crucial foodborne pathogens, such as *L. monocytogenes*, constitute a significant prerequisite for their application as an alternative increasing the safety of food products. Although the *in situ* antilisterial potential of *Pediococcus* spp. and its use in controlling growth of *L. monocytogenes* in meat products (Nieto-Lozano *et al.*, 2010; Kingcha *et al.*, 2012; De Azevedo *et al.*, 2020) and milk (Somkuti & Steinberg, 2009) is well studied, little scientific information is available on the antimicrobial activity against other members of genus *Listeria* as well as on the kinetics of the antimicrobial effect on these pathogens during *in vitro* co-cultivation.

Therefore, this research aimed to investigate the antimicrobial activity of three *Pediococcus* strains (*P. pentosaceus*, *P. acidilactici* and *P. damnosus*) against three *Listeria* spp. (*L. monocytogenes*, *L. innocua* and *L. ivanovii*) during co-cultivation under static growth conditions, and to determine the growth characteristics and basic kinetic parameters that could facilitate the selection of appropriate *Pediococcus* strains for production of safe fermented foods.

### MATERIAL AND METHODS

#### Microorganisms

Three *Pediococcus* strains (*P. pentosaceus* ATCC 33316, *P. acidilactici* NBIMCC 1603 and *P. damnosus* ATCC 29358) and three *Listeria* strains (*L. monocytogenes* NBIMCC 8632, *L. innocua* ATCC 33090 and *L. ivanovii* ATCC 19119) from the collection of the Department of Microbiology at the University of Food Technologies – Plovdiv, Bulgaria, were used in the experiments.

*Pediococcus* strains were cultured in MRS broth, while *Listeria* strains were cultured on BHI agar at 37°C for 24 h, and then stored at 4°C until use. Before the experiment, the strains were activated by propagation in MRS broth and BHI broth, respectively, and incubation at 37°C for 24 h.

**Culture media**

**LAPTg10 broth.** LAPTg10 broth was prepared according to the manufacturer’s (Laboratorios Conda S.A., Spain) prescription: 45 g of LAPTg10 base (containing 15 g peptone, 10 g yeast extract, 10 g tryptone and 10 g glucose) was dissolved in 1 L of deionized water (pH 6.6-6.8), and then 1mL Tween 80 (Sigma-Aldrich) was added. The medium was autoclaved at 121°C for 20 min.

**de Man, Rogosa and Sharpe (MRS) broth.** MRS broth was prepared by the manufacturer’s (Merck, Germany) prescription: 55.2 g of MRS broth (containing 10 g peptone, 5 g yeast extract, 10 g beef extract, 20 g glucose, 2 g potassium phosphate, 5 g sodium acetate, 0.2 g magnesium sulphate, 0.05 g manganese sulfate, 1 g Tween 80 and 2 g ammonium citrate) was dissolved in 1 L of deionized water (pH 6.4), and then the medium was autoclaved at 121°C for 15 min.

**de Man, Rogosa and Sharpe (MRS) agar.** This medium was prepared by the following prescription: 55.2 g of MRS broth (Merck, Germany) was dissolved in 1 L of deionized water (pH 6.4), and then 15 g of agar (Sigma-Aldrich) was added. The medium was autoclaved at 121°C for 15 min.

**Brain heart infusion (BHI) broth.** This medium was prepared according to the manufacturer’s (Oxoid, UK) prescription: 37 g of BHI broth (containing 12.5 g calf brain extract, 5 g beef heart extract, 10 g peptone, 5 g sodium chloride, 2 g glucose and 2.5 g disodium hydrogen phosphate) was dissolved in 1 L of deionized water (pH 7.4), and then the medium was autoclaved at 121°C for 15 min.

**Brain heart infusion (BHI) agar.** This medium was prepared by the following prescription: 37 g of BHI broth (Oxoid, UK) was dissolved in 1 L of deionized water (pH 7.4), and then 15 g of agar was added. The medium was autoclaved at 121°C for 15 min.

**Experimental design**

The determination of the antimicrobial activity of pediococci against *Listeria* strains was performed according to the method described by **Cholakov et al. (2017)** using fresh, 24-hour cultures of the studied microorganisms. In the experimental procedure, 0.5 mL of *Pediococcus* strain, 0.5 mL of *Listeria* strain and 9 mL of LAPTg10 broth (pre-determined as suitable for cultivation of both microorganisms) were mixed in sterile tubes. For preparation of the controls, 0.5 mL of the relevant *Pediococcus* strain with 9.5 mL of LAPTg10 broth and 0.5 mL of the relevant *Listeria* strain with 9.5 mL of LAPTg10 broth were mixed. Co-cultivation of pediococci and *Listeria* strains was carried out under static growth conditions in a thermostat at 37°C for 72 h. Samples at the 0th, 12th, 24th, 36th, 48th, 60th and 72nd h were taken and monitoring of the changes in the titratable acidity and the number of viable cells of pediococci and *Listeria* strains was conducted.

**Determination of titratable acidity**

The titratable acidity was determined by the titration method. For this purpose, 2.5 mL of each sample (mixtures and controls) was taken and mixed with 5 mL of distilled water. Then, a few drops of phenolphthalein as an indicator were added, and the titration was performed by 0.1 N NaOH until the appearance of a pale pink color persisting for over 1 min. The results were calculated as the mean value of three consecutive experiments and expressed as Torner degrees (°T). One Torner degree (°T) is equal to the amount of 0.1 N NaOH used to neutralize the equivalent amount of organic acid contained in 100 mL of culture medium (**Cholakov et al., 2017**).

**Determination of the viable cells counts of *Pediococcus* and *Listeria* strains**

Ten-fold serial dilutions of each sample (mixtures and controls) in sterile 0.5% NaCl were prepared. The determination of the number of viable cells of pediococci was performed by the spread plate method on MRS-agar. The determination of the number of viable *Listeria* cells was done by the spread plate method on BHI-agar. The Petri plates were incubated at 37°C for 3 days until appearance of single colonies. The results were expressed as colony-forming units (cfu)/cm<sup>3</sup> (**Cholakov et al., 2017**).

**Mathematical modelling and determination of basic kinetic parameters**

A modified logistic curve model (1) was used to determine the growth kinetics. The kinetics of death is modelled by an equation describing the kinetics of first-order chemical reactions (2), since the number of pathogens killed per unit time as a result of lactic acid bacteria-released metabolic products with antimicrobial activity (organic acids and bacteriocins) is proportional to the initial amount of viable cells.

$$\frac{dX}{d\tau} = \left(\mu X - \frac{\mu}{X_k} X^2\right)^n \Rightarrow \mu \left(1 - \frac{X}{X_k}\right)^n X \tag{1}$$

$$\frac{dX}{d\tau} = -kX \Rightarrow X = X_0 e^{-k\tau} \tag{2}$$

where:  $\mu$  – maximum specific growth rate, h<sup>-1</sup>;  $X_0$ ,  $X$  and  $X_k$  – initial, current and final viable cells counts, cfu/cm<sup>3</sup>;  $k$  – death rate constant, h<sup>-1</sup>;  $n$  – parameter showing the influence of the composition of the culture medium and the metabolic products released by the studied microorganisms (*Pediococcus* spp. and *Listeria* spp.) on the cells and their growth;  $\tau$  – time, h<sup>-1</sup>.

The differential equations were solved by the 4-th order Runge-Kuta method. To identify the kinetic parameters of the models, the Solver function in MS Office Excel 2010 software was used. The parameters of the model were determined by minimizing the square of the difference between the experimental data and those obtained from the model (**Choi et al., 2014**).

**Statistical analysis**

Data from triplicate experiments were processed with MS Office Excel 2010 software using statistical functions to determine the standard deviation (± SD) and maximum estimation error at significance levels  $\alpha < 0.05$ .

**RESULTS AND DISCUSSION**

The dynamics of changes in *Pediococcus* and *Listeria* viable cells counts during the process of single and co-cultivation at 37 ± 1°C is presented in **Figure 1A-C**; **Figure 3A-C** and **Figure 5A-C**. The kinetic parameters of the mathematical models used are shown in **Table 1**.

**Table 1** Kinetic parameters of the mathematical models used

Microorganism / mixture	Kinetic parameters			
	Model 1			Model 2
	$\mu_{max}$ , h <sup>-1</sup>	$X_k$ , cfu/cm <sup>3</sup>	$n$	$k$ , h <sup>-1</sup>
<i>P. pentosaceus</i> (control)	0.127	12.48	0.9242	-
<i>P. acidilactici</i> (control)	0.122	10.85	1.1711	-
<i>P. damnosus</i> (control)	0.074	10.85	1.1551	-
<i>L. monocytogenes</i> (control)	0.108	10.74	0.8775	-
<i>L. innocua</i> (control)	0.308	12.43	0.9824	-
<i>L. ivanovii</i> (control)	0.292	12.40	1.2096	-
<i>P. pentosaceus</i> + <i>L. monocytogenes</i>	0.121	11.71	1.0876	-
<i>P. pentosaceus</i> + <i>L. innocua</i>	0.124	11.46	1.1262	-
<i>P. pentosaceus</i> + <i>L. ivanovii</i>	0.123	12.00	1.1436	-
<i>L. monocytogenes</i> + <i>P. pentosaceus</i>	0.096	10.00	1.4401	0.337
<i>L. innocua</i> + <i>P. pentosaceus</i>	0.094	11.79	1.3748	0.568
<i>L. ivanovii</i> + <i>P. pentosaceus</i>	0.105	11.20	1.6705	0.546
<i>P. acidilactici</i> + <i>L. monocytogenes</i>	0.114	10.34	1.2045	-
<i>P. acidilactici</i> + <i>L. innocua</i>	0.115	10.66	1.2787	-
<i>P. acidilactici</i> + <i>L. ivanovii</i>	0.118	10.25	1.1528	-
<i>L. monocytogenes</i> + <i>P. acidilactici</i>	0.094	10.00	1.5385	0.588
<i>L. innocua</i> + <i>P. acidilactici</i>	0.092	10.15	1.6657	0.637
<i>L. ivanovii</i> + <i>P. acidilactici</i>	0.100	10.95	1.5082	0.632
<i>P. damnosus</i> + <i>L. monocytogenes</i>	0.060	10.15	1.6151	-
<i>P. damnosus</i> + <i>L. innocua</i>	0.061	10.81	1.5657	-
<i>P. damnosus</i> + <i>L. ivanovii</i>	0.065	10.74	1.5543	-
<i>L. monocytogenes</i> + <i>P. damnosus</i>	0.099	9.68	1.4256	0.159
<i>L. innocua</i> + <i>P. damnosus</i>	0.091	9.90	1.4063	0.084
<i>L. ivanovii</i> + <i>P. damnosus</i>	0.089	9.81	1.4034	0.060

$\mu_{max}$  – maximum specific growth rate, h<sup>-1</sup>;  $X_k$  – final viable cells count, cfu/cm<sup>3</sup>;  $n$  – parameter showing the influence of the composition of the culture medium and the metabolic products released by the studied microorganisms (*Pediococcus* spp. and *Listeria* spp.) on the cells and their growth;  $k$  – death rate constant, h<sup>-1</sup>.

During the single (independent) cultivation of *P. pentosaceus*, *P. acidilactici* and *P. damnosus*, high maximum specific growth ( $\mu_{max}$ ) rates were observed. The strains *P. pentosaceus* and *P. acidilactici* were characterized by commensurable  $\mu_{max}$ , which had values of 0.127 and 0.122 h<sup>-1</sup>, respectively. A lower  $\mu_{max}$  value was observed for the strain *P. damnosus* (0.074 h<sup>-1</sup>). The highest viable cells count for *P. pentosaceus* when cultivated singly was observed on the 24th h from the

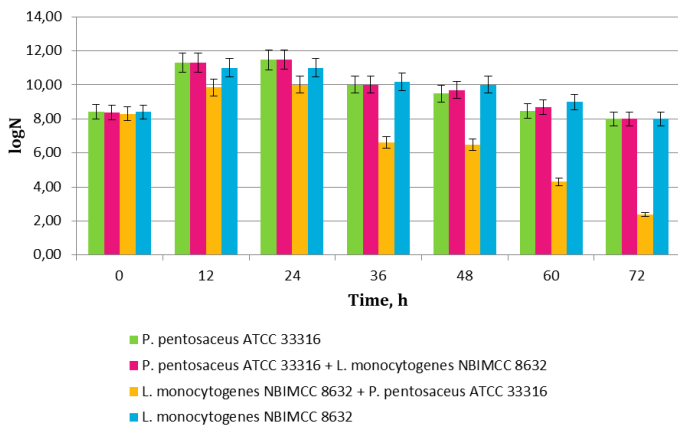
beginning of the process (11.46 logN), while for the strains *P. acidilactici* and *P. damnosus* it was reached on the 48th h and took values of 11.00 logN and 10.78 logN, respectively. These values were close to the highest viable cells count predicted by the mathematical model – 12.48 logN, 10.85 logN and 10.85 logN for *P. pentosaceus*, *P. acidilactici* and *P. damnosus*. After the 24th h for *P. pentosaceus* and after the 48th h for *P. acidilactici* and *P. damnosus*, a gradual reduction of viable cells for the three strains was observed, reaching final values of 8.00 logN, 9.30 logN and 9.20 logN for *P. pentosaceus*, *P. acidilactici* and *P. damnosus*, respectively. The parameter *n* for the strain *P. pentosaceus* was 0.9242, while for the other two strains it was 1.1711 and 1.1551, which demonstrated that the changes they cause in the culture medium as a result of their metabolic activity influenced their growth (Table 1).

A similar trend was observed during the single (independent) cultivation of the pathogens. Relatively high  $\mu_{max}$  rates were observed for the three test microorganisms – 0.108 h<sup>-1</sup> for *L. monocytogenes*, 0.308 h<sup>-1</sup> for *L. innocua* and 0.292 h<sup>-1</sup> for *L. ivanovii*. The highest viable cells counts for the three *Listeria* strains were 11.00 logN, 12.58 logN and 11.00 logN, whose values were in agreement with the experimentally predicted by the model – 10.74 logN, 12.43 logN and 12.40 logN for *L. monocytogenes*, *L. innocua* and *L. ivanovii*, respectively. *L. monocytogenes* and *L. innocua* reached the highest viable cells counts on the 12th h from the beginning of the cultivation. After the 24th h, their number began to decrease gradually, reaching value of 8.00 logN for both strains by the end of the process. The strain *L. ivanovii* reached the highest viable cells count on the 36th h from the beginning of the cultivation, after that a gradual reduction was observed, reaching value of 7.48 logN by the end of the process. The parameter *n* for the strains *L. monocytogenes* and *L. innocua* was 0.8775 and 0.9824, which indicated that the strains grow without being affected by changes in the composition of the culture medium as a result of their metabolic activity. In contrast, for *L. ivanovii* this parameter was 1.2096, which showed that environmental changes influenced the pathogen growth, but nevertheless it demonstrated a relatively high  $\mu_{max}$  rate (Table 1).

**Antimicrobial activity of *P. pentosaceus* against *L. monocytogenes*, *L. innocua* and *L. ivanovii* during co-cultivation at 37 ± 1°C**

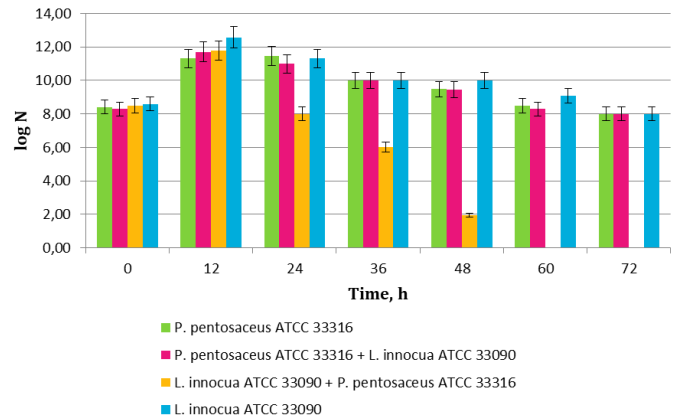
During co-cultivation of *P. pentosaceus* with *L. monocytogenes*, *L. innocua* and *L. ivanovii*, a slight reduction in the  $\mu_{max}$  rates of the three pathogens was observed, taking values of 0.121, 0.124 and 0.123 h<sup>-1</sup>, respectively, which were close to the controls. On the 24th h of co-cultivation of *P. pentosaceus* with the three *Listeria* strains, the highest viable cells counts (11.45 logN) were close to the experimentally defined by the model – 11.71 logN, 11.46 logN and 12.00 logN, followed by a gradual decrease in the viable cells count of *P. pentosaceus*. On the 72th h of co-cultivation, the viable cells count of *P. pentosaceus* was the same as the control – 8.00 logN. The parameter *n* increased for *P. pentosaceus* when co-cultured with the three pathogens and took values of 1.0876, 1.1262 and 1.1436, which demonstrated that the strain was slightly affected by the presence of *Listeria* spp. in the culture medium and released metabolites (Table 1).

During the co-cultivation of *L. monocytogenes*, *L. innocua* and *L. ivanovii* with *P. pentosaceus*, a reduction in the  $\mu_{max}$  rates for all three pathogens was observed, whose values were 0.096, 0.094 and 0.105 h<sup>-1</sup>, respectively. The highest viable cells counts of *L. monocytogenes*, *L. innocua* and *L. ivanovii* in the mixed populations were 10.69 logN, 12.02 logN and 11.60 logN, whose values were close to the experimentally determined by the model – 10.00 logN, 11.79 logN and 11.20 logN, respectively. *L. monocytogenes* reached the highest viable cells count in the mixed population on the 24th h, and then a reduction of the pathogen viable cells was observed (death rate constant of 0.337 h<sup>-1</sup>), as by the end of the cultivation process a value of 2.38 logN was detected (Figure 1A).

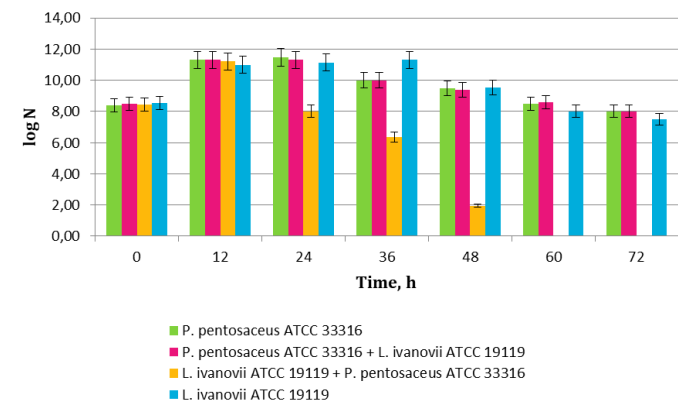


**Figure 1A** Survival of *P. pentosaceus* and *L. monocytogenes* cells during single and co-cultivation at 37 ± 1°C

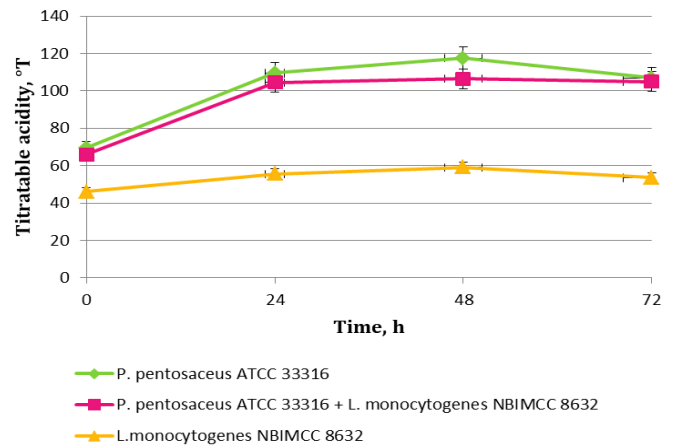
*L. innocua* and *L. ivanovii* reached the highest viable cells counts on the 12th h from the beginning of the co-cultivation, after that they began to decrease, and on the 60th h no viable cells of both pathogens were detected (Figure 1B and Figure 1C). The death rates for *L. innocua* and *L. ivanovii* were 0.568 and 0.546 h<sup>-1</sup>, respectively. The parameter *n* took values of 1.4401, 1.3748 and 1.6705 for *L. monocytogenes*, *L. innocua* and *L. ivanovii*, respectively, which showed a strong sensitivity of the three pathogens to the presence of the strain *P. pentosaceus* and antimicrobial metabolites released into the culture medium (Table 1). The reduction of *Listeria* spp. viable cells was due to the increasing titratable acidity of the culture medium in the three mixed populations, reaching 107°T on the 48th h and remained almost constant until the end of co-cultivation process (Figure 2A, Figure 2B and Figure 2C).



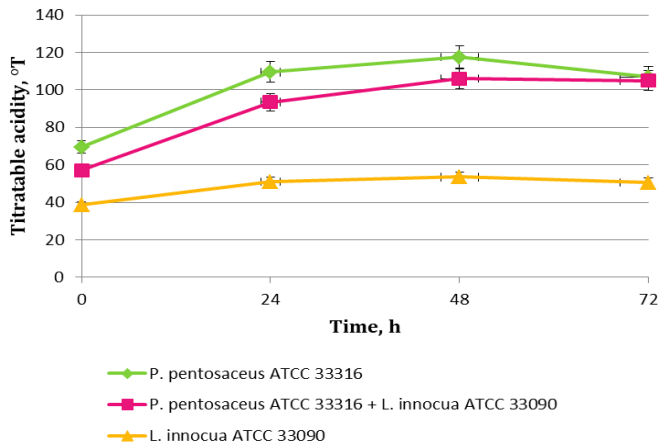
**Figure 1B** Survival of *P. pentosaceus* and *L. innocua* cells during single and co-cultivation at 37 ± 1°C



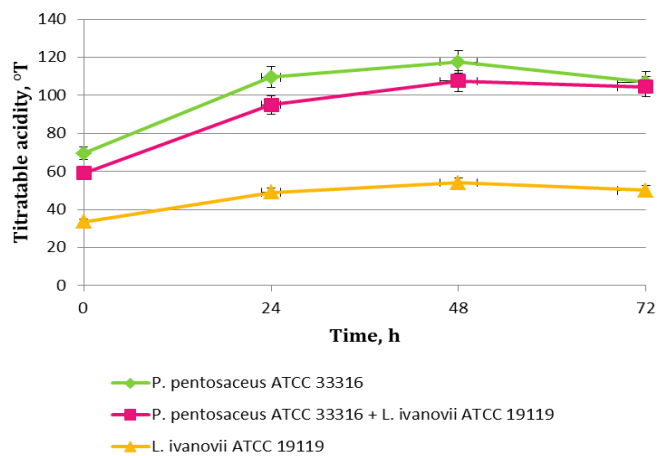
**Figure 1C** Survival of *P. pentosaceus* and *L. ivanovii* cells during single and co-cultivation at 37 ± 1°C



**Figure 2A** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. pentosaceus* and *L. monocytogenes* at 37 ± 1°C



**Figure 2B** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. pentosaceus* and *L. innocua* at 37 ± 1°C



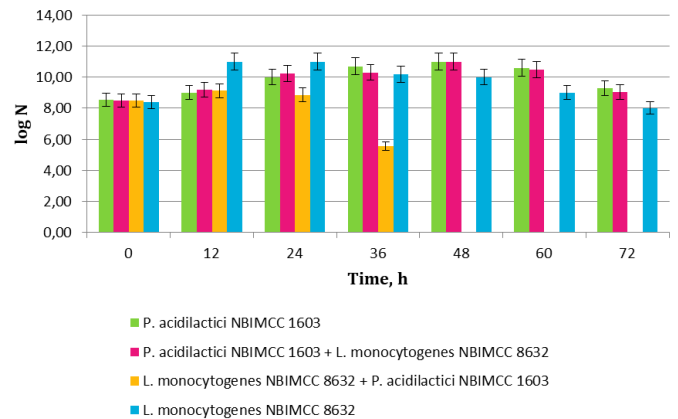
**Figure 2C** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. pentosaceus* and *L. ivanovii* at 37 ± 1°C

**Antimicrobial activity of *P. acidilactici* against *L. monocytogenes*, *L. innocua* and *L. ivanovii* during co-cultivation at 37 ± 1°C**

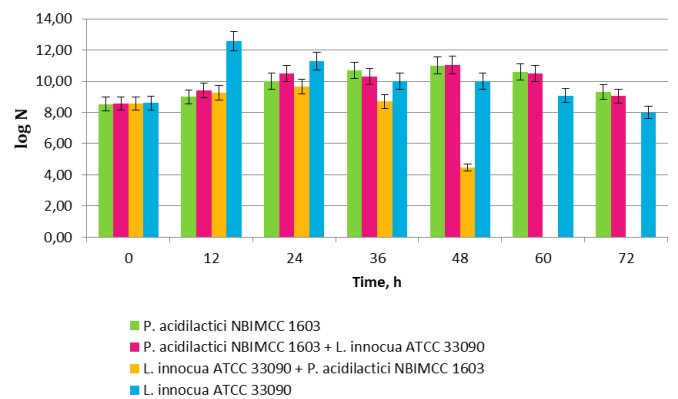
During the co-cultivation of *P. acidilactici* with *L. monocytogenes*, *L. innocua* and *L. ivanovii*, a slight reduction in the  $\mu_{max}$  rates was observed, which took values of 0.114, 0.115 and 0.118 h<sup>-1</sup> for the three tested pathogens, respectively. The highest viable cells counts of *P. acidilactici* in the mixed populations (11.00 logN) were achieved on the 48th h of co-cultivation process (with *L. monocytogenes*) and on the 24th h (with *L. innocua* and *L. ivanovii*), respectively, whose values were close to the experimentally predicted by the mathematical model – 10.34 logN, 10.66 logN and 10.25 logN. The parameter *n* increased for *P. acidilactici* when co-cultivated with the three pathogens and assumed values of 1.2045, 1.2787 and 1.1528, which showed that the strain was affected by the presence of *Listeria* and the released metabolites in the culture medium (Table 1).

During the co-cultivation of *L. monocytogenes*, *L. innocua* and *L. ivanovii* with *P. acidilactici*, a reduction in the  $\mu_{max}$  rates was observed for all three pathogens with values of 0.094, 0.092 and 0.100 h<sup>-1</sup>, respectively. The highest viable cells counts of the three pathogens in the mixed populations were 9.11 logN, 9.66 logN and 10.23 logN, respectively, which were close to the experimentally determined by the model – 10.00 logN, 10.15 logN and 10.95 logN. The strain *L. monocytogenes* reached the highest viable cells count in the mixed population on the 12th h of co-cultivation, after which an intense decrease in the pathogen viable cells with a high death rate of 0.588 h<sup>-1</sup> was observed, and on the 48th h no viable cells of *L. monocytogenes* were detected (Figure 3A). The highest viable cells counts of the strains *L. innocua* and *L. ivanovii* were reached on the 24th h from the beginning of the co-cultivation, after which high death rates of 0.637 and 0.632 h<sup>-1</sup> were observed, and on the 60th h no viable cells of *L. innocua* and *L. ivanovii* were detected (Figure 3B and Figure 3C). The increase in the parameter *n* (1.5385, 1.6657 and 1.5082 for *L. monocytogenes*, *L. innocua* and *L. ivanovii*) showed a high sensitivity of the three tested pathogens to the presence of *P. acidilactici* and produced antimicrobial metabolites in the culture medium (Table 1). This was in compliance with the high values of titratable acidity in the mixed populations of the strain *P. acidilactici* with *L. monocytogenes*, *L. innocua* and *L. ivanovii*

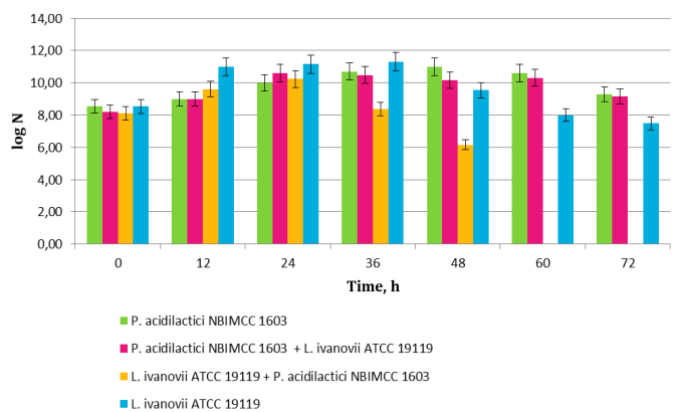
reaching 159°T, 179°T and 155°T, respectively (Figure 4A, Figure 4B and Figure 4C), and retained constant levels until the end of the process.



**Figure 3A** Survival of *P. acidilactici* and *L. monocytogenes* cells during single and co-cultivation at 37 ± 1°C



**Figure 3B** Survival of *P. acidilactici* and *L. innocua* cells during single and co-cultivation at 37 ± 1°C



**Figure 3C** Survival of *P. acidilactici* and *L. ivanovii* cells during single and co-cultivation at 37 ± 1°C

**Antimicrobial activity of *P. damnosus* against *L. monocytogenes*, *L. innocua* and *L. ivanovii* during co-cultivation at 37 ± 1°C**

During the co-cultivation of *P. damnosus* with the three *Listeria* strains, a reduction in the  $\mu_{max}$  rates was observed, reaching values of 0.060, 0.061 and 0.065 h<sup>-1</sup> for *L. monocytogenes*, *L. innocua* and *L. ivanovii*, respectively. The strain *P. damnosus* reached the highest viable cells counts on the 48th h of co-cultivation with values of 10.00, 10.48 and 10.18 logN in the mixed populations with the three *Listeria* strains. These experimental values were similar to the theoretically predicted by the model – 10.15, 10.81 and 10.74 logN, respectively. After 48 h from the beginning of the co-cultivation, a reduction in the viable cells of *P. damnosus* was detected, but the final viable cells count of the strain was close to that of the control

and varied in the range of 9.00 to 9.20 logN. During the co-cultivation of *P. dammosus* with *L. monocytogenes*, *L. innocua* and *L. ivanovii*, an increase in the parameter *n* (1.6151, 1.5657 and 1.5543) was observed, indicating that *P. dammosus* was affected by the presence of *Listeria* spp. in the culture medium (Table 1).

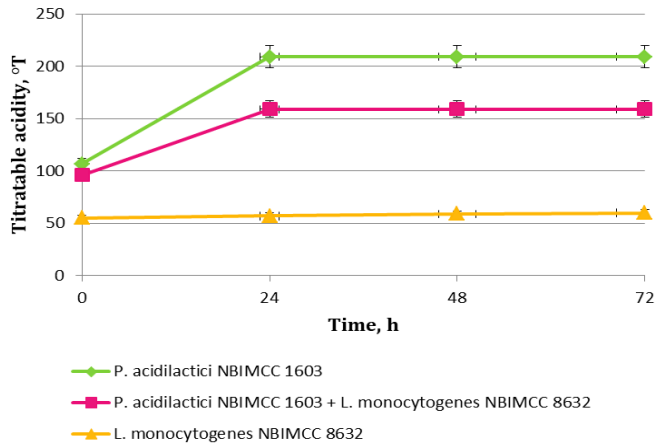


Figure 4A Changes in titratable acidity of the culture medium during single and co-cultivation of *P. acidilactici* and *L. monocytogenes* at 37 ± 1°C

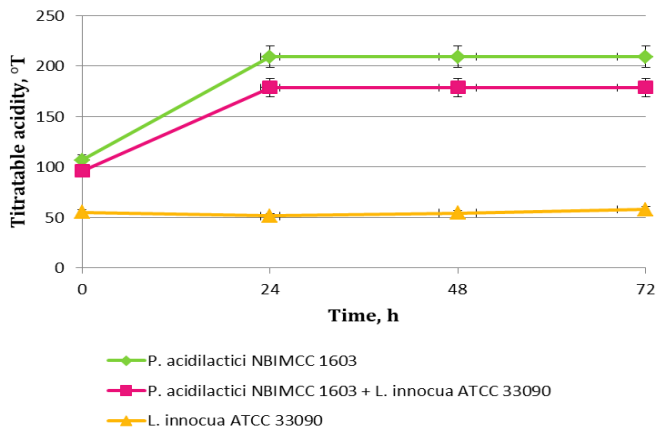


Figure 4B Changes in titratable acidity of the culture medium during single and co-cultivation of *P. acidilactici* and *L. innocua* at 37 ± 1°C

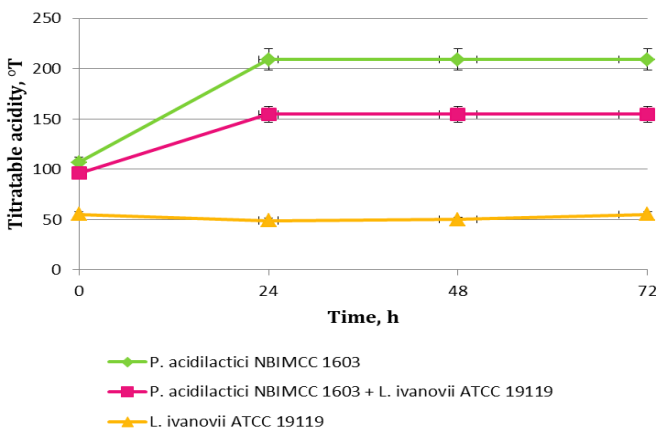


Figure 4C Changes in titratable acidity of the culture medium during single and co-cultivation of *P. acidilactici* and *L. ivanovii* at 37 ± 1°C

The co-cultivation of *L. monocytogenes*, *L. innocua* and *L. ivanovii* with *P. dammosus* showed a decrease in the  $\mu_{max}$  rates – 0.099, 0.091 and 0.089 h<sup>-1</sup>, respectively. The highest viable cells counts of *L. monocytogenes*, *L. innocua* and *L. ivanovii* were observed on the 36th h of co-cultivation with *P. dammosus*, reaching values of 9.48, 9.85 and 9.83 logN in the mixed populations. These values were very close to the theoretically predicted by the model – 9.68, 9.90 and 9.81

logN, respectively. After the 36th h of co-cultivation of *L. monocytogenes*, *L. innocua* and *L. ivanovii* with *P. dammosus*, all studied strains maintained relatively constant viable cells counts. On the 72nd h of co-cultivation, a weak reduction of *L. monocytogenes*, *L. innocua* and *L. ivanovii* viable cells was observed, reaching 7.00, 8.41 and 8.78 logN by the end of the process (Figure 5A, Figure 5B and Figure 5C). The increase of parameter *n* (1.4256, 1.4063 and 1.4034) showed a strong competition for substrates between the different species in mixed populations (Table 1). Based on the pathogens viable cell counts and values of parameter *n*, it can be assumed that *P. dammosus* exerts a bacteriostatic rather than a bactericidal effect on the studied *Listeria* strains. The titratable acidity of the culture medium in the mixed populations reached 83°T, 79°T and 79°T by the 72nd h of co-cultivation (Figure 6A, Figure 6B and Figure 6C).

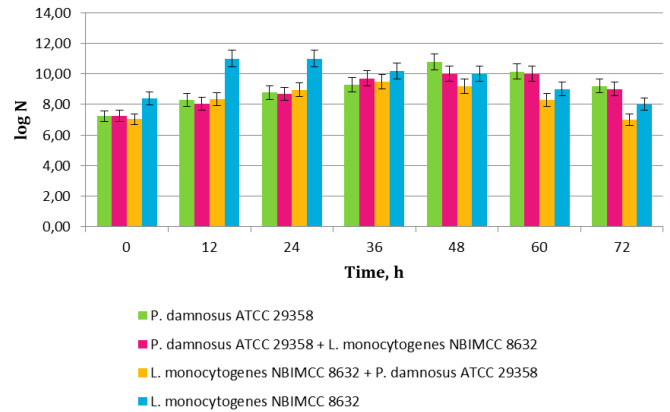


Figure 5A Survival of *P. dammosus* and *L. monocytogenes* cells during single and co-cultivation at 37 ± 1°C

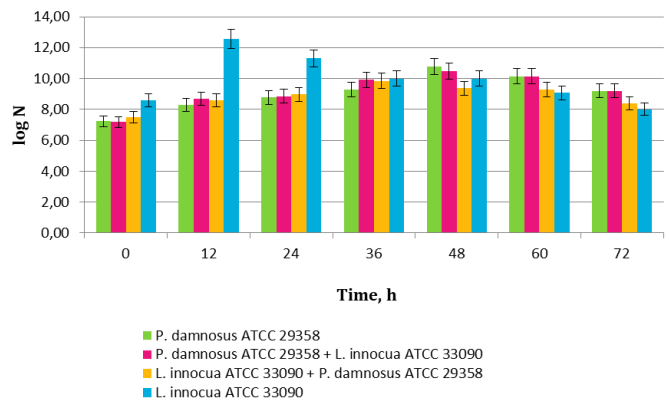


Figure 5B Survival of *P. dammosus* and *L. innocua* cells during single and co-cultivation at 37 ± 1°C

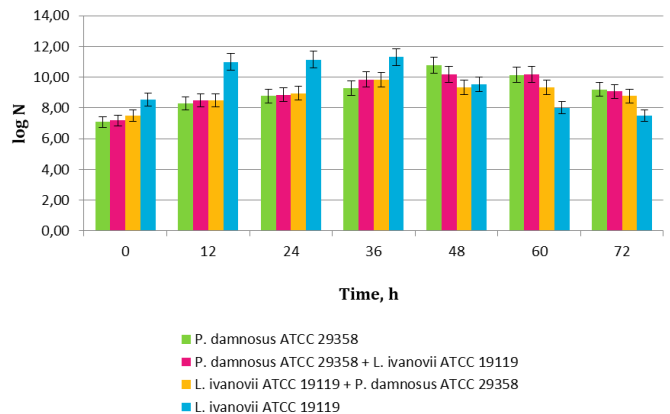
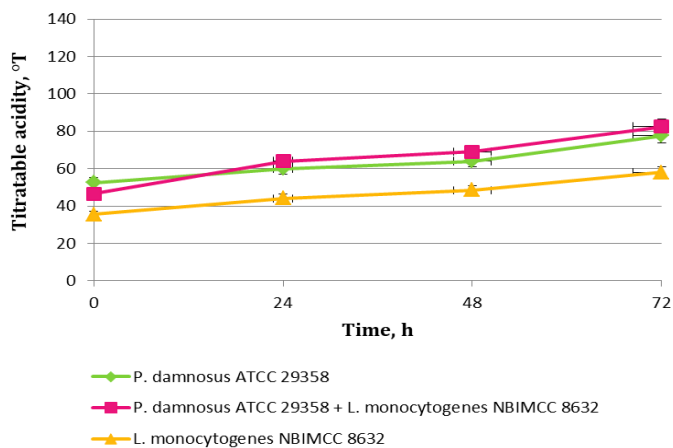
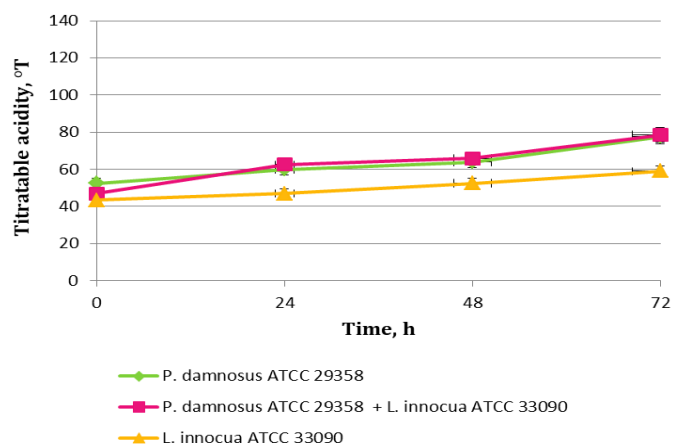


Figure 5C Survival of *P. dammosus* and *L. ivanovii* cells during single and co-cultivation at 37 ± 1°C



**Figure 6A** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. damnosus* and *L. monocytogenes* at 37 ± 1°C

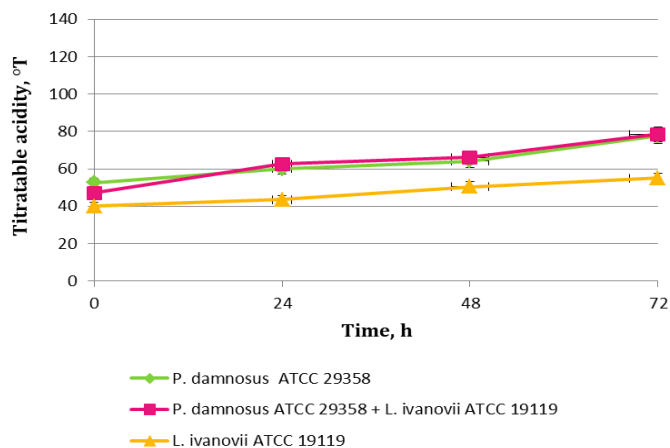
In recent decades, numerous studies have been carried out to prove the *in vitro* inhibitory effect of pediococci and bacteriocin production on *Listeria* spp. Altuntas et al. (2010) studied the growth parameters and bacteriocin production by *P. acidilactici* 13 and determined that the strain possessed high antimicrobial activity against *L. monocytogenes* and ability to grow in a wide temperature range (25–50°C) and high saline concentrations (up to 10% NaCl). The authors stated that bacteriocin obtained by *P. acidilactici* 13 was sensitive to proteinase K and exhibited its maximal activity at pH 6.0 and temperature of 37°C. Cavicchioli et al. (2017) isolated *P. pentosaceus* ST65ACC from Brazilian type of cheese, and the results demonstrated that the bacteriocin produced by this strain had a strong antagonistic effect on *L. monocytogenes* and *L. innocua*, and completely inactivated the cells of both pathogens within 12 h. The antimicrobial activity of obtained bacteriocins was affected by proteolytic enzymes, but they remained active after being exposed to a wide pH range (2.0 to 10.0) for 2 h. Engelhardt et al. (2015) found that *P. acidilactici* HA6111-2 had a pronounced antimicrobial effect on two tested serotypes of *L. monocytogenes* (isolated from cheese and beef, respectively) and one of *L. innocua*. The authors also pointed out that this strain exhibited a good growth and strong antilisterial effect even under stress conditions (pH 3.5–8.5 and 7.5% NaCl).



**Figure 6B** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. damnosus* and *L. innocua* at 37 ± 1°C

Fermented products of animal origin may contain different foodborne pathogens and spoilage microorganisms depending on the manufacture conditions. Therefore, the selection of appropriate starter cultures possessing high antimicrobial potential is of great importance in order to be effective against undesirable microflora when applied in the food matrix. Some researchers recommend the use of *P. acidilactici* to control *L. monocytogenes* in vacuum-packed sausages, after proving that the strains *P. acidilactici* JBL1095 and *P. acidilactici* LB42 effectively inhibited *Listeria* sp. growth on the surface of experimentally contaminated fresh sausages at refrigeration conditions and room temperature (Degnan et al., 1992). The antimicrobial efficacy of *P. acidilactici* against *L. monocytogenes* was also confirmed in a series of experiments in fresh chicken meat. Goff et al. (1996) found that raw chicken meat treated with pediocin AcH produced by the strain *P. acidilactici* H retained its antilisterial activity, both under refrigeration and after heat treatment, and this fact was most likely due to binding of pediocin to meat proteins. The study of Murray & Richard (1997) demonstrated the strong

antimicrobial effect of the bacteriocin pediocin AcH from *P. acidilactici* on *L. innocua* in artificially infected fresh pork meat. Ladha & Jeevaratnam (2020) isolated and characterized a 4.6 kDa bacteriocin from *P. pentosaceus* LJR1 and found that it possess inhibitory effect on *L. monocytogenes* leading to reduction of about 1 log unit in treated white leg shrimp during storage for 7 days at 4°C. Besides antilisterial effect, some *Pediococcus* spp. isolates from fermented Turkish “sucuk” sausage were shown to possess significant antimicrobial activity against *Staphylococcus aureus* (Cosansu et al., 2007). The results from these and many other studies have demonstrated that *Pediococcus* strains and bacteriocins synthesized by them are of considerable interest in food systems, especially fermentation processes, and they can be successfully used in the biopreservation of various food products, improving their quality, shelf life and safety.



**Figure 6C** Changes in titratable acidity of the culture medium during single and co-cultivation of *P. damnosus* and *L. ivanovii* at 37 ± 1°C

## CONCLUSION

To our knowledge, this is the first study to investigate the antimicrobial activity of *Pediococcus* spp. against *Listeria* spp. during co-cultivation, which determines the growth characteristics and main kinetic parameters of the used strains. Based on the obtained results, we can summarize that *P. pentosaceus* ATCC 33316 possessed a bacteriostatic effect on *L. monocytogenes* NBIMCC 8632 and a bactericidal effect on *L. innocua* ATCC 33090 and *L. ivanovii* ATCC 19119. *P. acidilactici* NBIMCC 1603 demonstrated a strong bactericidal effect on the three *Listeria* strains, which are among the most common contaminants in food products of animal origin. In contrast, the strain *P. damnosus* ATCC 29358 showed only a bacteriostatic effect on the three tested pathogens. Therefore, the strains *P. pentosaceus* ATCC 33316 and *P. acidilactici* NBIMCC 1603 can be successfully applied in the food industry as starter cultures and natural preservatives in order to enhance the quality, shelf life and safety of various food products.

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## REFERENCES

Adesulu-Dahunsi, A. T., Sanni, A. I., & Jeyaram, K. (2021). Diversity and technological characterization of *Pediococcus pentosaceus* strains isolated from Nigerian traditional fermented foods. *LWT-Food Science and Technology*, 140, 110697. <https://dx.doi.org/10.1016/j.lwt.2020.110697>

Altuntas, E. G., Cosansu, S., & Ayhan K. (2010). Some growth parameters and antimicrobial activity of a bacteriocin-producing strain *Pediococcus acidilactici* 13. *International Journal of Food Microbiology*, 141(1-2), 28-31. <https://dx.doi.org/10.1016/j.ijfoodmicro.2010.04.024>

Beneduce, L., Spano, G., Vernile, A., Tarantino, D., & Massa, S. (2004). Molecular characterization of lactic acid populations associated with wine spoilage. *Journal of Basic Microbiology*, 44(1), 10-16. <https://dx.doi.org/10.1002/jobm.200310281>

Cai, Y., Kumai, S., Ogawa, M., Benno, Y., & Nakase, T. (1999). Characterization and identification of *Pediococcus* species isolated from forage crops and their application for silage preparation. *Applied and Environmental Microbiology*, 65(7), 2901-2906. <https://dx.doi.org/10.1128/aem.65.7.2901-2906.1999>

Cavicchioli, V. Q., Camargo, A. C., Todorov, S. D., & Nero, L. A. (2017). Novel bacteriocinogenic *Enterococcus hirae* and *Pediococcus pentosaceus* strains with antilisterial activity isolated from Brazilian artisanal cheese. *Journal of Dairy Science*, 100(4), 2526-2535. <https://dx.doi.org/10.3168/jds.2016-12049>

Choi, M., Saeed Al-Zahrani, M., & Lee, S.Y. (2014). Kinetic model-based feed-forward controlled fedbatch fermentation of *Lactobacillus rhamnosus* for the production of lactic acid from Arabic date juice. *Bioprocess and Biosystems Engineering*, 37, 1007-1015. <https://dx.doi.org/10.1007/s00449-013-1071-7>

Cholakov, R., Tumbarski, Y., Yanakieva, V., Dobrev, I., Salim, Y., & Denkova, Z. (2017). Antimicrobial activity of *Leuconostoc lactis* strain BT17, isolated from

- a spontaneously fermented cereal beverage (boza). *Journal of Microbiology, Biotechnology and Food Sciences*, 7(1), 47-49. <https://dx.doi.org/10.15414/jmbfs.2017.7.1.47-49>
- Cosansu, S., Kuleasan, H., Ayhan, K., & Materon, L. (2007). Antimicrobial activity and protein profiles of *Pediococcus* spp. isolated from Turkish "sucuk". *Journal of Food Processing and Preservation*, 31(2), 190-200. <https://dx.doi.org/10.1111/j.1745-4549.2007.00122.x>
- De Azevedo, P. O. S., Mendonça, C. M. N., Seibert, L., Domínguez, J. M., Converti, A., Gierus, M., & Oliveira, R. P. S. (2020). Bacteriocin-like inhibitory substance of *Pediococcus pentosaceus* as a biopreservative for *Listeria* sp. control in ready-to-eat pork ham. *Brazilian Journal of Microbiology*, 51, 949-956. <https://dx.doi.org/10.1007/s42770-020-00245-w>
- Degnan, A. J., Yousef, A. E., & Luchansky, J. B. (1992). Use of *Pediococcus acidilactici* to control *Listeria monocytogenes* in temperature-abused vacuum-packaged wieners. *Journal of Food Protection*, 55(2), 98-103. <https://dx.doi.org/10.4315/0362-028X-55.2.98>
- Engelhardt, T., Albano, H., Kiskó, G., Mohácsi-Farkas, C., & Teixeira, P. (2015). Antilisterial activity of bacteriocinogenic *Pediococcus acidilactici* HA6111-2 and *Lactobacillus plantarum* ESB 202 grown under pH and osmotic stress conditions. *Food Microbiology*, 48, 109-115. <https://dx.doi.org/10.1016/j.fm.2014.11.015>
- Franz, C. M. A. P., Endo, A., Abriouel, H., Reenen, C. A. V., Gálvez, A., & Dicks, L. M. T. (2014). The genus *Pediococcus*. In: *Lactic Acid Bacteria: Biodiversity and Taxonomy*, Wilhelm H. Holzappel, Brian J. B. Wood, Eds., 359-376. <https://dx.doi.org/10.1002/9781118655252.ch21>
- Goff, J. H., Bhunia, A. K., & Johnson, M. G. (1996). Complete inhibition of low levels of *Listeria monocytogenes* on refrigerated chicken meat with pediocin AcH bound to heat-killed *Pediococcus acidilactici* cells. *Journal of Food Protection*, 59(11), 1187-1192. <https://dx.doi.org/10.4315/0362-028X-59.11.1187>
- Gurira, O. Z., & Buys, E. M. (2005). Characterization and antimicrobial activity of *Pediococcus* species isolated from South African farm-style cheese. *Food Microbiology*, 22(2-3), 159-168. <https://dx.doi.org/10.1016/j.fm.2004.08.001>
- Iijima, K., Suzuki, K., Asano, S., Kuriyama, H., & Kitagawa, Y. (2007). Isolation and identification of potential beer-spoilage *Pediococcus inopinatus* and beer-spoilage *Lactobacillus backi* strains carrying the horA and horC gene clusters. *Journal of the Institute of Brewing*, 113(1), 96-101. <https://dx.doi.org/10.1002/j.2050-0416.2007.tb00262.x>
- Jamuna, M., & Jeevaratnam, K. (2004). Isolation and partial characterization of bacteriocins from *Pediococcus* species. *Applied Microbiology and Biotechnology*, 65(4), 433-439. <https://dx.doi.org/10.1007/s00253-004-1576-8>
- Kingcha, Y., Tosukhowong, A., Zendo, T., Roytrakul, S., Luxanani, P., Chareonpomsook, K., Valyasevi, R., Sonomoto, K., & Visessanguan, W. (2012). Anti-listeria activity of *Pediococcus pentosaceus* BCC 3772 and application as starter culture for Nham, a traditional fermented pork sausage. *Food Control*, 25(1), 190-196. <https://dx.doi.org/10.1016/j.foodcont.2011.10.005>
- Ladha, G., & Jeevaratnam, K. (2020). Characterization of purified antimicrobial peptide produced by *Pediococcus pentosaceus* LJR1, and its application in preservation of white leg shrimp. *World Journal of Microbiology and Biotechnology*, 36(5), 72. <https://dx.doi.org/10.1007/s11274-020-02847-w>
- Mora, D., Musacchio, F., Fortina, M. G., Senini, L., & Manachini, P. L. (2003). Autolytic activity and pediocin-induced lysis in *Pediococcus acidilactici* and *Pediococcus pentosaceus* strains. *Journal of Applied Microbiology*, 94(4), 561-570. <https://dx.doi.org/10.1046/j.1365-2672.2003.01868.x>
- Murray, M., & Richard, J. A. (1997). Comparative study of the antilisterial activity of nisin A and pediocin AcH in fresh ground pork stored aerobically at 5°C. *Journal of Food Protection*, 60(12), 1534-1540. <https://dx.doi.org/10.4315/0362-028X-60.12.1534>
- Nghe, D., & Nguyen, T. (2014). Characterization of antimicrobial activities of *Pediococcus pentosaceus* Vtc-B-601. *Journal of Applied Pharmaceutical Science*, 4(05), 061-064. <https://dx.doi.org/10.7324/JAPS.2014.40511>
- Nieto-Lozano, J. C., Reguera-Useros, J. I., Peláez-Martínez, M. C., Sacristán-Pérez-Minayo, G., Gutiérrez-Fernández, Á. J., & la Torre, A. H. (2010). The effect of the pediocin PA-1 produced by *Pediococcus acidilactici* against *Listeria monocytogenes* and *Clostridium perfringens* in Spanish dry-fermented sausages and frankfurters. *Food Control*, 21(5), 679-685. <https://dx.doi.org/10.1016/j.foodcont.2009.10.007>
- Pal, V., Pal, A., Patil, M., Ramana, K. V., & Jeevaratnam, K. (2010). Isolation, biochemical properties and application of bacteriocins from *Pediococcus pentosaceus* isolates. *Journal of Food Processing and Preservation*, 34(6), 1064-1079. <https://dx.doi.org/10.1111/j.1745-4549.2009.00438.x>
- Plessas, S., Mantzourani, I., & Bekatorou, A. (2020). Evaluation of *Pediococcus pentosaceus* SP2 as starter culture on sourdough bread making. *Foods*, 9(1), 77. <https://dx.doi.org/10.3390/foods9010077>
- Porto, M. C. W., Kuniyoshi, T. M., Azevedo, P. O. S., Vitolo, M., & Oliveira, R. P. S. (2017). *Pediococcus* spp.: An important genus of lactic acid bacteria and pediocin producers. *Biotechnology Advances*, 35(3), 361-374. <https://dx.doi.org/10.1016/j.biotechadv.2017.03.004>
- Sakamoto, K., & Konings, W. N. (2003). Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology*, 89(2-3), 105-124. [https://dx.doi.org/10.1016/s0168-1605\(03\)00153-3](https://dx.doi.org/10.1016/s0168-1605(03)00153-3)
- Skariyachan, S., & Govindarajan, S. (2019). Biopreservation potential of antimicrobial protein producing *Pediococcus* spp. towards selected food samples in comparison with chemical preservatives. *International Journal of Food Microbiology*, 291, 189-196. <https://dx.doi.org/10.1016/j.ijfoodmicro.2018.12.002>
- Somkuti, G. A., & Steinberg, D. H. (2009). Pediocin production in milk by *Pediococcus acidilactici* in co-culture with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Journal of Industrial Microbiology & Biotechnology*, 37(1), 65-69. <https://dx.doi.org/10.1007/s10295-009-0648-2>