Control of *Listeria monocytogenes* by bacteriocin-producing *Pediococcus acidilactici* 13 and its antimicrobial substance in a dry fermented sausage sucuk and in turkey breast

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Summary

This study evaluated control of *Listeria monocytogenes* during sucuk (a dry fermented sausage) ripening and storage of a sliced turkey breast product with *Pediococcus acidilactici* 13, which had been originally isolated from naturally fermented sucuk. When *P. acidilactici* 13 was used as a starter culture for sucuk production, *L. monocytogenes* counts decreased by 3.32 log CFU·g^{-1} during the 8-day ripening period, whereas the reduction in control samples was 1.37 log CFU·g^{-1} ($P < 0.05$). Treatment of turkey breast slices with partially purified substance of *P. acidilactici* 13 resulted in an immediate reduction by 1.03 log CFU·cm^{-2} ($P < 0.05$). It was concluded that *P. acidilactici* 13 could be useful as a protective culture for control of *L. monocytogenes* in particular in fermented meat products. The antimicrobial substance produced by this strain could only reduce the contamination by *L. monocytogenes* in a non-fermented meat product.

Keywords

Pediococcus acidilactici; *Listeria monocytogenes*; fermented sausage; turkey breast; protective culture

Listeria monocytogenes is one of the most important food-borne pathogens, being often associated with meat and poultry products [1]. It is important to reduce both the incidence and levels of *L. monocytogenes* in foods [2].

Sucuk, a dry fermented meat product of Turkey, is prepared by mixing ground beef, tallow, NaCl, saccharose, spices and other additives such as sodium nitrate and ascorbic acid, and stuffing into natural or artificial casings before fermentation [3]. Fermentation is based on the natural flora present in the raw material, while use of starter cultures for sucuk production is not common in Turkey except for big firms. It has been reported that naturally fermented sucuk samples may show large variations in their chemical and microbiological characteristics, and often they may be found unacceptable in appearance and in chemical and microbiological properties [4]. Furthermore, producers offer unripened products for sale within 2–3 days after stuffing. The concern is that during this time period, *L. monocytogenes* may grow in the product, at least during the first few days of fermentation. Surveys [5–7] have found 7–23% prevalence of *L. monocytogenes* in sucuk samples offered for sale. Therefore, as sucuk may constitute a human health risk if contaminated and consumed without cooking or undercooked, it would be useful to develop approaches for control of *L. monocytogenes* during preparation of the product. Appropriately low water activity, acidic pH, presence of nitrate, nitrite and NaCl are known to contribute to pathogen control and prolong shelf-life in fermented and other types of sausage products. Furthermore, use of effective starter cultures could also contribute to product safety through uniform and consistent fermentation resulting in formation of antimicrobial metabolites, including acids and bacteriocins [8–10].

Contamination of ready-to-eat (RTE) meat and poultry products (e.g., deli meats) with *L. monocytogenes* is also a significant public health concern. Such products have been linked to fatal listeriosis outbreaks and major product recalls

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in North America [11]. The high risk for listeriosis associated with deli meats is partly attributed to certain product characteristics (e.g., nutrients, pH, water activity) that create the potential for *L. monocytogenes* growth to high numbers during distribution and retail storage before consumption. The primary sources of contamination of deli meats, and other RTE meat and poultry products, with *L. monocytogenes* are food processing equipment and other surfaces during processes that follow the cooking step, such as slicing and repackaging [12]. In United States, regulation requires that meat processors control the pathogen in such products by applying physical intervention and/or using growth inhibitors in product formulations or as post-processing surface treatments, to inactivate, *L. monocytogenes* and/or prevent or suppress growth of survivors during storage [13]. The meat industry is thus in need of research data to help them meet these regulatory requirements.

The objectives of this study were to evaluate the potential antilisterial effect of a *Pediococcus acidilactici* strain in sucuk when used as a starter culture, and on RTE uncured turkey breast slices treated with a partially purified substance of that strain.

MATERIALS AND METHODS

Bacterial cultures

Preliminary studies verified the antimicrobial properties of *Pediococcus acidilactici* 13 isolated from sucuk in a previous study by our laboratory [14, 15]. The substance was also found to be heat-stable and of proteinaceous nature, being sensitive to trypsin but not to pepsin (unpublished data). Ten *L. monocytogenes* strains (558, N-7150, NA-1, N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, R2-765) were from the collection of Colorado State University, Department of Animal Sciences, Pathogen Reduction Laboratory (Fort Collins, Colorado, USA) [16]. *L. monocytogenes* strains N1-225, N1-227, R2-500, R2-501, R2-763, R2-764 and R2-765 were kindly provided by Dr. Martin Wiedmann (Cornell University, Ithaca, New York, USA) [17]. Preliminary studies using the agar spot and well diffusion assays [18] indicated that *P. acidilactici* 13 inhibited the growth of all the *L. monocytogenes* strains, except strain 558.

Effect of sucuk ingredients on antimicrobial activity

An initial study tested *P. acidilactici* 13 for activity in the presence of sucuk ingredients. Individual ingredients were added into test tubes containing 20 ml of de Man – Rogosa – Sharpe broth (MRS; Difco Becton Dickinson, Sparks, Maryland, USA) at levels used for sucuk production (ascorbic acid 0.05%, red pepper 1.0%, black pepper 0.6%, cumin 1.0%, NaCl 2.0%, saccharose 0.6%, garlic 2.5%, $NaNO_3$ 0.05%). Additionally, all of the ingredients combined at the above concentrations were added into a flask that contained 100 ml of MRS broth. Also, tubes of MRS broth were prepared without the addition of any of the ingredients to serve as controls. All tubes and the flask were autoclaved and then inoculated with the *P. acidilactici* 13 culture. After incubation at 30 °C for 24 h, the cultures were centrifuged at 3214 ×*g* for 10 min at 4 °C and supernatants were sterilized by microfiltration (pore size, 0.2 μm). The antimicrobial activity of the supernatants was tested by the well diffusion assay [18] against a mixture of nine *L. monocytogenes* strains (N-7150, NA-1, N1-225, N1-227, R2-500, R2-501, R2-763, R2-764, R2-765).

Sucuk production

A nine-strain *L. monocytogenes* mixture (i.e., excluding strain 558 which was resistant to the antimicrobial metabolite(s) of *P. acidilactici* 13; this strain will be valuable in future studies evaluating mechanisms of antimicrobial activity) was used for inoculation of the sucuk batter. Each strain was propagated at 30 °C during 24 h on slants of TSAYE - tryptic soy agar (Difco, Becton Dickinson) supplemented with 0.6% yeast extract (Acumedia, Lansing, Michigan, USA) and maintained at 4 °C. Before each experiment, strains were individually activated and subcultured at 30 °C during 24 h in 10 ml tryptic soy broth (Difco Becton Dickinson) supplemented with 0.6% yeast extract (TSBYE). The cultures of all strains were mixed equally and centrifuged at 4629 ×*g* for 10 min at 4 °C. The supernatant was removed and the pellet was washed with phosphate-buffered saline (PBS; pH 7.4, containing 0.2 g KH_2PO_4, 1.5 g $Na_2HPO_4.7H_2O$, 0.8 g NaCl and 0.2 g KCl in 1l distilled water). After centrifugation, the supernatant was discarded and the pellet was resuspended in PBS to the original volume. The mixed *L. monocytogenes* culture was appropriately diluted with PBS to obtain a level of 4 log CFU·g^{-1} in the sucuk batter.

After propagating *P. acidilactici* 13 twice in MRS broth at 30 °C for 24 h, 10 ml of the culture was centrifuged and washed with PBS as described above. After washing, the supernatant was discarded and the pellet was resuspended in 25 ml PBS. The target level of the *Pediococcus* culture in the sucuk batter was 7 log CFU·g^{-1}.

Beef (5% fat) and tallow (15%) were ground to particles of 4 mm and divided into two batches, each weighing 2 kg. One batch was inoculated with 50 ml of the diluted *L. monocytogenes* culture (4 log CFU·g^{-1} inoculation level), and the other batch with 25 ml of the diluted *L. monocytogenes* culture (4 log CFU·g^{-1} inoculation level) together with 25 ml of the *Pediococcus* culture (7 log CFU·g^{-1} inoculation level). After inoculation, each batch was mixed (KitchenAid, Professional 600, St. Joseph, Michigan, USA) for 3 min, and sucuk ingredients (0.05% ascorbic acid, 1.0% red pepper, 0.6% black pepper, 1.0% cumin, 2.0% NaCl, 0.6% saccharose, 2.5% garlic, and 0.05% $NaNO_3$) were added into the ground meat and mixed for additional 3 min. Batters were extruded into natural hog casings (38–40 mm diameter; DeWied International, San Antonio, Texas, USA), and ripening was carried out at (24 ± 2) °C and 90–95% relative humidity (RH) for 3 days, then at (22 ± 2) °C and 80–85% RH for 5 days. Two replicate experiments were conducted. Three samples were analysed from each group immediately after stuffing and then on days 1, 2, 4, 6 and 8 of the fermentation period.

Treatment of turkey breast slices

A partially purified substance of the *P. acidilactici* 13 was evaluated against *L. monocytogenes* surface-inoculated on uncured turkey breast slices to confirm antimicrobial activity and evaluate the potential for use of the culture in products other than sucuk. The culture preparation was produced [19] by culturing the *Pediococcus* strain twice in MRS broth (30 °C; 24 h) and then inoculating 1 l of MRS broth and incubating at 30 °C for 24 h. The culture was then heated at 70 °C for 30 min to inactivate cells and enzymes, and it was centrifuged at 4629 ×*g* for 20 min at 4 °C. After adjusting pH to 6.5 with 1 mol·l^{-1} NaOH, the supernatant was filter-sterilized (0.2 μm), and ammonium sulphate was gradually added to achieve 60% saturation. The material was kept overnight at 4 °C with gentle stirring (1.66 Hz), and then centrifuged at 18514 ×*g* for 20 min at 4 °C. The sediment at the bottom and floating solid material were collected and dissolved in 1 l of phosphate buffer (67 mmol·l^{-1} KH_2PO_4 and Na_2PO_4; pH 6.5). Antimicrobial activity of this solution was determined to be 6 400 AU·ml^{-1} using the method of Biswas et al. [20]. The partially purified antimicrobial substance preparation was used to treat inoculated turkey breast slices.

Uncured (without nitrite), cooked, ready-to-eat turkey breast, obtained directly from a commercial manufacturer, consisted of turkey breast, turkey broth, NaCl, modified food starch, saccharose, carrageenan, sodium phosphate and flavourings. Before inoculation with *L. monocytogenes* to a target inoculum level of 2–3 log CFU·cm^{-2}, turkey breast was sliced (to a thickness of approximately 3 mm) and slices were cut into 25 cm^2 pieces (5 × 5 cm), and divided in two groups. The first group was inoculated with a mixture of the nine sensitive *L. monocytogenes* strains (as used previously in the sucuk experiment), and the other with *L. monocytogenes* strain 558, which was found to be resistant to the antimicrobial metabolite(s) of the *P. acidilactici* 13 in the preliminary studies. A volume of 0.1 ml of *L. monocytogenes* inoculum (5 log CFU·ml^{-1}) was deposited on one side of each slice and was spread over the entire surface with a sterile bent glass rod. Inoculated slices were left to stand at 4 °C for 15 min for inoculum attachment. The same procedure was repeated for the other side of each slice. Then, half of the inoculated slices from each group were put in presterilized glass containers (15–16 slices per tray) and 225 ml of the antimicrobial substance preparation (6 400 AU·ml^{-1}) was poured over the slices. Slices were maintained in the solution for 2 min with shaking, and then drained in sterile strainers for 15 min. The untreated samples were used as controls. Immediately after completion of exposure to each treatment, samples, comprised of two slices, were placed into vacuum bags (15 × 22 cm, 0.1 mm standard barrier, Nylon/PE vacuum pouch; Koch, Kansas City, Missouri, USA), vacuum packaged at 10.7 mPa (Hollymatic, Countryside, Illinois, USA), and stored at 12 °C for 10 days. Two replicates were conducted, and three samples per treatment were periodically analysed during storage to determine survival/growth of bacterial populations.

Microbiological analyses

Sucuk samples of 10 g from each treatment were placed in sterile plastic bags (Whirl-Pak, Nasco, Modesto, California, USA) at 0, 1, 2, 4, 6 and 8 days of fermentation, mixed with 90 ml of maximum recovery diluent (MRD; 0.1% peptone and 0.85% NaCl), and homogenized for 2 min (Masticator, IUL Instruments, Barcelona, Spain). Appropriate serial dilutions were made in 0.1% buffered peptone water (Difco Becton Dickinson) and plated by spreading 0.1 ml on duplicate plates of TSAYE for total plate count (TPC) enumeration (25 °C, 72 h) and on PALCAM agar (Difco Becton Dickinson) for *L. monocytogenes* enumeration (30 °C, 48 h). Lactic acid bacteria were enumerated by pour plating 1 ml of sample on MRS agar of pH 5.5 (adjusted with HCl) in order

to make the medium more selective for isolation of lactic acid bacteria; the colonies were counted manually after incubation at 25 °C for 72 h.

Turkey breast samples were analysed for microbiological counts at 0, 3, 6, and 10 days of storage. Slices of each treatment were transferred into individual Whirl-Pak bags, mixed with 50 ml of MRD, shaken 30 times [21], and appropriate serial dilutions of the rinsate were spread-plated on duplicate plates of TSAYE and PALCAM agar. Microbiological counts were expressed as log CFU·cm^{-2}, given that *L. monocytogenes* was surface-inoculated on turkey breast slices. The total surface area of each sample was 100 cm^2 (25 cm^2/side × 2 sides × 2 slices/bag).

Water activity and pH determination

Water activity (a_w) of sucuk samples was determined with an AquaLab water activity meter (Model series 3; Decagon Devices, Pullman, Washington, USA) on each sampling day. Water activity values of turkey breast samples were also determined, but only on day 0 of storage. The pH of samples was measured after microbiological analysis by immersing a glass electrode (Denver Instruments, Arvada, Colorado, USA) into the Whirl-Pak bag containing the diluted (in MRD) sample. The pH values were determined for all product samples at each sampling day.

Statistical analyses

Data relating to microbial counts, pH and water activity values for sucuk and turkey breast samples were subjected to ANOVA using SPSS version 14.0 for Windows (Chicago, Illinois, USA). "Treatment × Time" and "Treatment × Group" interactions were tested. Duncan's multiple range test was used to separate means at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Effect of sucuk ingredients on antimicrobial activity of *P. acidilactici* 13

In general, the culture of *P. acidilactici* 13 maintained the antilisterial activity in broth containing sucuk ingredients, although its activity was somewhat lower in the presence of red pepper (Tab. 1). However, when the sucuk ingredients were added as a mixture, antimicrobial activity was similar to that in the absence of the ingredients (control). This indicates that the non-meat ingredients should not interfere with the *Pediococcus* culture during sucuk fermentation.

Antimicrobial activity of *P. acidilactici* against *L. monocytogenes* in sucuk

L. monocytogenes is a food-borne pathogen and human listeriosis outbreaks have been associated with contaminated meat and poultry products [11, 12]. Although naturally occurring starter cultures may contribute to *L. monocytogenes* control in sausage fermentation, their antimicrobial activity may be lower than desired in a meat matrix because of variability in the food micro-environment and variability in natural microbial contamination associated with batches of meat and other ingredients [22]. For this reason, it may be preferable to select starter cultures that are adapted to the meat environment, and are able to compete in the meat system in order to be able to produce antimicrobial metabolites in situ, and consequently be more suitable as food-preserving microorganisms compared to cultures isolated from other environments [19]. Therefore, in this study, we used an antimicrobial substance-producing *Pediococcus* strain previously isolated from sucuk, which could potentially be useful as a starter culture for its production.

There are several reports on the antilisterial effects of *Pediococcus* spp. isolated from Turkish fermented sucuk [4, 23, 24]. Also, some researchers [8, 25] have investigated the behavior of *L. monocytogenes* during sucuk fermentation using bacteriocin-producer starter cultures. As far as we could determine, however, this is the first report on the antilisterial effects of a *Pediococcus* sp., isolated from a naturally fermented sucuk sample, during sucuk ripening.

Tab. 1. Antimicrobial activity of *P. acidilactici* 13 against *L. monocytogenes* after growth in MRS broth containing sucuk ingredients.

Ingredients	Inhibition zones [mm]
Control	21.5 ± 2.1^{a}
Mixture (all ingredients)	21.0 ± 1.4^{ab}
Ascorbic Acid	19.0 ± 1.4^{ab}
NaCl	22.0 ± 0.0^{a}
Saccharose	20.5 ± 0.7^{ab}
$NaNO_3$	20.0 ± 0.0^{ab}
Red Pepper	17.0 ± 1.4^{b}
Black Pepper	19.0 ± 0.0^{ab}
Cumin	18.0 ± 1.4^{ab}
Garlic	19.0 ± 0.0^{ab}

Values are expressed as mean ± standard deviation. Means having different lowercase symbols are significantly different ($P < 0.05$).

Tab. 2. *Listeria monocytogenes,* lactic acid bacteria and total plate counts determined during sucuk fermentation.

Day	*Listeria monocytogenes* counts* [log CFU·g^{-1}]		Lactic acid bacteria counts* [log CFU·g^{-1}]		Total plate counts [log CFU·g^{-1}]	
	Control	Treatment	Control	Treatment	Control	Treatment
0	4.69 ± 0.11^{Aa}	4.53 ± 0.08^{Aa}	2.37 ± 0.39^{Aa}	7.06 ± 0.10^{Ab}	5.61 ± 1.26^{Aa}	7.45 ± 0.54^{Ab}
1	3.66 ± 0.09^{Ba}	3.46 ± 0.23^{Ba}	4.24 ± 1.28^{Ba}	7.06 ± 0.11^{Ab}	5.61 ± 0.60^{Aa}	7.10 ± 0.07^{Ab}
2	3.77 ± 0.27^{Ba}	3.13 ± 0.14^{Bb}	5.88 ± 1.22^{Ca}	7.45 ± 0.37^{Bb}	6.17 ± 1.12^{ABa}	7.24 ± 0.36^{Ab}
4	3.72 ± 0.23^{Ba}	2.06 ± 0.24^{Cb}	7.33 ± 0.88^{Ca}	9.07 ± 0.10^{Cb}	7.08 ± 0.97^{BCa}	9.02 ± 0.06^{Bb}
6	3.50 ± 0.29^{Ba}	1.70 ± 0.73^{Cb}	7.15 ± 1.39^{Ca}	9.15 ± 0.25^{Cb}	7.74 ± 0.87^{Ca}	9.17 ± 0.30^{Bb}
8	3.32 ± 0.90^{Ba}	1.21 ± 0.30^{Db}	7.20 ± 1.35^{Ca}	9.09 ± 0.19^{Cb}	7.41 ± 1.08^{BCa}	9.05 ± 0.24^{Bb}

Values are expressed as mean ± standard deviation.
Any two means in each bacterial counts in the same row having different lowercase symbols are significantly different ($P < 0.05$).
Any two means in the same column having different uppercase symbols are significantly different ($P < 0.05$).
* – "Treatment × Time" interaction was significant ($P < 0.05$). Control – ripened without added starter culture, Treatment – ripened with *P. acidilactici* 13.

Results from this study showed that *L. monocytogenes* counts in the control sucuk samples were reduced ($P < 0.05$) by 1.03 log CFU·g^{-1} on the first day of the fermentation period, while they remained constant ($P \geq 0.05$) during subsequent days of fermentation (Tab. 2). Similar findings were reported by EROL et al. [8] who found that *L. monocytogenes* counts in control sucuk samples (without starter culture) were reduced by 1 log during the initial several days of fermentation and, then, remained constant during maturation. In this study, the reduction of *L. monocytogenes* counts in the samples inoculated with *Pediococcus* sp. strain 13 was 3.32 log CFU·g^{-1} at the end of the 8-day fermentation period. Our results are comparable to LÜCKE [26], who studied the in situ effect of bacteriocins against *L. monocytogenes* in meat systems and found the reduction of *L. monocytogenes* counts by 1–2 log units compared to bacteriocin-negative controls.

Counts of lactic acid bacteria (LAB) increased ($P < 0.05$) from 2.37 log CFU·g^{-1} to 7.20 log CFU·g^{-1} and from 7.06 log CFU·g^{-1} to 9.09 log CFU·g^{-1} in control and *Pediococcus*-inoculated samples, respectively (Tab. 2). The differences in LAB counts between the two groups were significant ($P < 0.05$). Increases in TPC were similar to those of LAB counts in both treatments; from 5.61 log CFU·g^{-1} to 7.41 log CFU·g^{-1} and from 7.45 log CFU·g^{-1} to 9.05 log CFU·g^{-1} ($P < 0.05$), respectively (Tab. 2). The pH values of sucuk samples inoculated with the *Pediococcus* culture decreased from 5.54 to 4.92 ($P < 0.05$), while the pH values of control samples did not change ($P \geq 0.05$) during ripening (Tab. 3). During the 8-day fermentation period, the a_W values of

Tab. 3. The pH and a_w values of sucuk samples during fermentation.

Day	pH*		a_W	
	Control	Treatment	Control	Treatment
0	5.53 ± 0.03^{a}	5.54 ± 0.03^{Aa}	0.971 ± 0.003^{A}	0.968 ± 0.006^{A}
1	5.59 ± 0.01^{a}	5.58 ± 0.02^{Aa}	0.958 ± 0.004^{B}	0.960 ± 0.001^{AB}
2	5.59 ± 0.04^{a}	5.44 ± 0.06^{Bb}	0.952 ± 0.007^{B}	0.953 ± 0.003^{B}
4	5.60 ± 0.06^{a}	5.04 ± 0.01^{Cb}	0.929 ± 0.016^{C}	0.926 ± 0.017^{C}
6	5.56 ± 0.08^{a}	4.95 ± 0.03^{Db}	0.921 ± 0.002^{C}	0.917 ± 0.013^{C}
8	5.52 ± 0.15^{a}	4.92 ± 0.05^{Db}	0.887 ± 0.008^{D}	0.887 ± 0.013^{D}

Values are expressed as mean ± standard deviation.
* – "Treatment × Time" interaction for pH was significant statistically ($P < 0.05$). Control – ripened without added starter culture, Treatment – ripened with *P. acidilactici* 13.
Any two means in each bacterial counts in the same row having different lowercase symbols are significantly different ($P < 0.05$).
Any two means in the same column having different uppercase symbols are significantly different ($P < 0.05$).

control and treated samples decreased ($P < 0.05$) from 0.971 to 0.887 and from 0.968 to 0.887, respectively (Tab. 3); a_W values were not different ($P \geq 0.05$) between the two treatments.

Although LAB counts in the control samples reached 7.20 log CFU·g^{-1} at the end of the fermentation period, there was no significant reduction ($P \geq 0.05$) in the pH values (pH 5.52). These results suggest that the natural flora of the raw material could not produce enough lactic acid to reduce the pH. However, in the *Pediococcus* culture-inoculated samples, LAB counts reached a level of 9.09 log CFU·g^{-1} on day 8 of the fermentation, and the pH was lowered to a mean value of 4.92±0.05. Similarly, KAYA and GÖKALP [27] determined that the pH values of sucuk samples inoculated with starter culture were reduced to below 5.0 during first 3 days of fermentation, whereas the pH values of samples fermented without the starter culture remained between 5.38 and 5.46. FOEGEDING et al. [28] reported that effective reduction in *L. monocytogenes* populations was correlated with an adequate pH decrease (below 4.9) during the fermentation, and that in situ bacteriocin production enhanced the inhibition of *L. monocytogenes* during both fermentation and drying. This added inhibition would be especially important if a sufficiently low pH was not achieved during fermentation; for instance, if the initial pH value of raw meat was high. Therefore, *P. acidilactici* 13 used in this study may be considered as a potential starter culture, not only for its antilisterial metabolite(s), but also for its pH-lowering ability.

Antilisterial activity of partially purified substance on turkey breast slices

L. monocytogenes counts in 9-strain inoculated control samples (9 strains-control) of turkey breast slices and those immersed in the partially purified antimicrobial substance preparation (9 strains + substance preparation) were 2.69 log CFU·cm^{-2} and 1.66 log CFU·cm^{-2}, respectively, on day 0 of storage (Tab. 4). For control samples inoculated with the *Pediococcus* isolate-resistant *L. monocytogenes* strain 558 (558-control) and those immersed in the supernatant preparation (558 + supernatant preparation), initial pathogen counts were 2.48 log CFU·cm^{-2} and 2.18 log CFU·cm^{-2}, respectively. *L. monocytogenes* counts increased ($P < 0.05$) in all samples during storage at 12 °C for 10 days. However, counts of samples inoculated with the 9-strain composite and treated with the supernatant preparation remained lower than the other treatments ($P < 0.05$). Total plate counts

Tab. 4. Changes in *L. monocytogenes* counts on turkey breast slices stored at 12 °C.

Day	9 strains-control [log CFU·g^{-1}]	9 strains + antimicrobial substance preparation [log CFU·g^{-1}]	558-control [log CFU·g^{-1}]	558 + antimicrobial substance preparation [log CFU·g^{-1}]
0	2.69 ± 0.15^{Aa}	1.66 ± 0.16^{Ac}	2.48 ± 0.41^{Aab}	2.18 ± 0.20^{Ab}
3	6.72 ± 0.14^{Ba}	5.48 ± 0.46^{Bb}	6.59 ± 0.16^{Ba}	6.83 ± 0.46^{Ba}
6	7.41 ± 0.23^{Ca}	5.72 ± 0.11^{CDb}	7.73 ± 0.44^{Ca}	7.78 ± 0.51^{Ca}
10	7.61 ± 0.48^{Ca}	6.33 ± 0.97^{Db}	7.82 ± 0.42^{Ca}	7.99 ± 0.48^{Ca}

Values are expressed as mean ± standard deviation.
Any two means in the same row having different lowercase symbols are significantly different ($P < 0.05$). Any two means in the same column having different uppercase symbols are significantly different ($P < 0.05$).

Tab. 5. Changes in total plate counts (TPC) on turkey breast slices stored at 12 °C.

Day	9 strains-control [log CFU·g^{-1}]	9 strains + antimicrobial substance preparation [log CFU·g^{-1}]	558-control [log CFU·g^{-1}]	558 + antimicrobial substance preparation [log CFU·g^{-1}]
0	2.79 ± 0.11^{Aa}	1.82 ± 0.24^{Ab}	2.50 ± 0.34^{Ac}	2.25 ± 0.20^{Ac}
3	6.74 ± 0.05^{Ba}	5.86 ± 0.23^{Bb}	6.82 ± 0.26^{Ba}	7.02 ± 0.04^{Ba}
6	7.83 ± 0.25^{Ca}	8.00 ± 0.17^{Cab}	8.04 ± 0.18^{Cab}	8.18 ± 0.17^{Cb}
10	8.29 ± 0.13^{Da}	7.75 ± 0.52^{Cb}	8.27 ± 0.23^{Ca}	8.31 ± 0.12^{Ca}

Values are expressed as mean ± standard deviation.
Any two means in the same row having different lowercase symbols are significantly different ($P < 0.05$). Any two means in the same column having different uppercase symbols are significantly different ($P < 0.05$).

increased similarly in samples of all treatments, despite an initial reduction ($P < 0.05$) in counts of samples inoculated with the 9-strain composite and treated with the supernatant preparation (Tab. 5).

Day-0 a_W values of turkey breast samples from all treatments were in the range of 0.970–0.983, while pH values were 6.37–6.38 and decreased ($P < 0.05$) during storage at 12 °C reaching values of 5.72–5.81 on day-10 (data not shown). Differences in pH values among treatments were not statistically significant.

As indicated, the effectiveness of the partially purified antimicrobial substance (6400 AU·ml^{-1}) produced by *P. acidilactici* 13, applied as an external immersion solution against inoculated (2–3 log CFU·cm^{-2}) *L. monocytogenes*, was examined in a food system (i.e., uncured turkey breast slices). Immediately after treatment with the antimicrobial substance preparation, *L. monocytogenes* counts on the samples inoculated with the nine *Pediococcus* culture-sensitive *L. monocytogenes* strain mixture were reduced by 1.03 log CFU·cm^{-2} compared to untreated controls, while the *Pediococcus* culture-resistant *L. monocytogenes* strain 558 was reduced by only 0.30 log CFU·cm^{-2}. Even though *L. monocytogenes* counts on turkey breast samples inoculated with the 9-strain mixture and treated with the antimicrobial substance preparation remained lower than other treatments during storage (12 °C, 10 days), the pathogen grew well on this product, as previously reported [29] for sliced chicken and turkey stored at 4.4 °C. In previous studies, *L. monocytogenes* counts were reduced during storage at 5–6 °C on samples treated with reuterin [30] and pediocin AcH [31], while counts of untreated control samples remained at the inoculation level during the storage period. Unlike these previous studies, in our study, turkey breast samples were stored at an abusive temperature (12 °C). Therefore, it appears that treatment with the partially purified antimicrobial substance preparation may have an immediate effect against *L. monocytogenes*, and extent of further control of the pathogen may depend on the storage temperature.

Our results showed that treatment of turkey breast slices with the antimicrobial substance preparation was not completely inhibitory against *L. monocytogenes* during storage at 12 °C, even though it reduced levels of the pathogen and the samples had lower counts than other treatments throughout storage. Possible reasons for the lower effectiveness of the substance during storage of turkey breast samples could be its adsorption to fat and protein particles and potential inactivation by food additives, natural proteases or other compounds [9, 32]. However, its application may still be useful, in particular if applied in a combination with organic acid solutions, as demonstrated with nisin [33, 34].

CONCLUSIONS

This study provides data suggesting that *P. acidilactici* 13 could be used as a starter and protective culture in sucuk fermentation for control of *L. monocytogenes.* Since *P. acidilactici* 13 has been isolated from sucuk, it is well adapted to sucuk conditions and, consequently, can be considered as more suitable as a food-preserving microorganism for sucuk fermentation than others isolated from different environments. On the other hand, treatment of turkey breast slices with a partially purified antimicrobial substance of *P. acidilactici* 13 reduced, but did not prevent *L. monocytogenes* growth during storage at an abusive temperature of 12 °C. However, since the treatment reduced the populations of *L. monocytogenes*, it may be useful for reducing the *L. monocytogenes* risk in meat products which do not undergo a fermentation step, if used in combination with other intervention against *L. monocytogenes*.

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