Short communication: Antimicrobial potential of *Lactobacillus plantarum* strains isolated from Slovak raw sheep milk cheeses

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ABSTRACT

The objective of this study was to screen *Lactobacillus plantarum* strains isolated from the traditional Slovak raw sheep milk cheese for their inhibitory potential. Seventy-two strains were obtained from samples of raw sheep milk and raw sheep milk cheeses collected from April 2017 to September 2018, in 23 geographical areas of Eastern Slovakia, by inoculation of de Man, Rogosa, and Sharpe agar plates (Oxoid, Basingstoke, UK). Using both the genus- and species-specific PCR methods, 43 strains were identified as *Lactobacillus* spp., and 10 strains were confirmed as *Lb. plantarum*. First, the whole bacterial cultures of *Lb. plantarum* strains were tested by disc diffusion assay. All showed very good antibacterial activities against 6 selected foodborne pathogens, including *Escherichia coli*, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus cereus*. Then, cell-free neutralized supernatants and partially purified bacteriocins were prepared from the 4 *Lb. plantarum* strains that exhibited the best antibacterial potential, and they were tested the same way as the whole bacterial cultures. Seven of the 10 *Lb. plantarum* strains harbored the *plnEF* gene, 3 strains harbored the *plnD* gene, and 2 strains possessed both the *plnA* and *plnC* genes that encode the production of the respective plantaricins. The presence of both *plnR* and *plnL* genes was only detected in a single *Lb. plantarum* isolate. Based on the results of this study, 4 strains of *Lb. plantarum* appeared to be suitable candidates for further testing in the dairy manufacturing sector, particularly in the production of raw sheep milk products.

Key words: *Lactobacillus plantarum*, antimicrobial activity, plantaricin, sheep cheese

Short Communication

There is a worldwide, growing demand for fresh foods without preservatives and synthetic additives. Protective bacterial cultures, including bacteriocin-producing lactic acid bacteria (**LAB**), appear to be a promising alternative (Field et al., 2018). The use of probiotic cultures can help manufacturers ensure the quality and safety of ready-to-eat, fresh-tasting, extended-shelf-life, and minimally processed foods, without chemical preservatives (Hammami et al., 2019). Therefore, the need continues in Slovak and other European dairy industries to prepare new, natural, protective bacterial cultures ready for immediate use in milk processing, especially in the production of unpasteurized sheep milk products.

Samples of starter-free raw milk sheep cheeses collected from 23 different regions of Eastern Slovakia, including areas with protected geographical indication, were investigated for the presence of LAB. Seventy-two strains of gram-positive, rod-shaped, non-motile, and catalase-negative bacteria were obtained by inoculation of de Man, Rogosa, and Sharpe (**MRS**) agar plates (Oxoid, Basingstoke, UK). Using the genus-specific PCR, 43 strains were identified as *Lactobacillus* spp. The results of species-specific PCR confirmed 10 strains as *Lb. plantarum*, all obtained from raw sheep milk cheeses ripened for more than 4 d. The presence of *Lb. plantarum* in traditional Slovakian Bryndza cheese was also confirmed in other studies, indicating that this species participates in the cheese ripening process (Berta et al., 2009; Belicová et al., 2011; Chebeňová-Turcovská et al., 2011). Antimicrobial activities of *Lb. plantarum* strains against 6 selected foodborne pathogens were screened using disc diffusion assay. Details of all meth-

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Figure 1. Diameters of inhibition zones shown by selected indicator strains due to antimicrobial activity of *Lactobacillus plantarum* whole bacterial cultures (designated L) from Slovak raw sheep milk cheeses. CCM = Czech Collection of Microorganisms (Brno, Czech Republic).

ods, primers, and reference strains used in this study are available in Supplemental File S1 and Supplemental Table S1 (<https://doi.org/10.3168/jds.2019-17862>).

Antagonistic activity of *Lb. plantarum* whole bacterial cultures (**WBC**) against selected indicator strains is shown in Figure 1. All *Lb. plantarum* cultures exhibited visible antibacterial activity, especially against *Pseudomonas aeruginosa* and *Escherichia coli*. Viable cells of lactobacilli in the substrate may produce several antibacterial metabolites, such as lactic acid, acetic acid, hydrogen peroxide, carbon dioxide, diacetyl and ethanol, or bacteriocins, which are synthesized ribosomally (Tejero-Sariñena et al., 2012). The production of species-specific bacteriocins (plantaricins) has never been studied in the Slovak Republic.

Six *pln* locus genes were detected in the *Lb. plantarum* strains, and 6 of the 10 strains (L15, L16, L19, L20, L21, and L22) harbored the *plnEF* gene. Two strains

(L19 and L20) also showed the presence of *plnA*, *plnC*, and *plnD* genes, and another strain (L5) was positive for both *plnR* and *plnL* genes. However, none of the strains showed the presence of *plnJ*, *plnK*, *plnM*, *plnN*, or *plnB* genes, and 3 strains (L10, L17 and L18) did not contain any of the 11 *pln* genes tested. This does not exclude a possible production of other bacteriocin-like proteins. The presence of genes encoding synthesis of plantaricins in *Lb. plantarum* strains isolated from raw milk cheeses has been reported by other authors (Portilla-Vázquez et al., 2016; Azizi et al., 2017). Fernandes et al. (2017) suggested plantaricin-producing lactobacilli as candidates for biopreservation in artisanal raw milk cheese.

Based on the inhibitory effect of WBC and the presence of bacteriocin genes, 4 *Lb. plantarum* strains were selected for further testing of the cell-free neutralized supernatants (**CFNS**). As shown in Table 1, significant

Table 1. Antimicrobial activity of the cell-free neutralized supernatants of *Lactobacillus plantarum* strains (L5, L19, L20, and L22) isolated from Slovak raw sheep milk cheeses

Indicator strain ¹	Diameter of inhibition zone, ² mm			
	L5	L19	L20	L22
<i>Salmonella</i> Enteritidis CCM 4420	0	0	9.6	8.3
<i>Listeria monocytogenes</i> CCM 5676	0	0	10.0	11.3
<i>Pseudomonas aeruginosa</i> CCM 3955	0	0	10.5	0
<i>Staphylococcus aureus</i> CCM 5756	0	0	12.4	8.1
M6	50.9	55.3	43.8	27.5
M8	14.6	40.1	40.8	0
M12	22.6	31.6	35.5	32.7
M16	36.5	0	0	24.0

¹Indicator reference strains = CCM (Czech Collection of Microorganisms, Brno, Czech Republic); wild *Staphylococcus aureus* strains = M.

²Entry of 0 indicates diameter ≤ 8 mm.

Table 2. Antibacterial activity of partially purified bacteriocins produced by *Lactobacillus plantarum* strains (L5, L19, L20, and L22) isolated from Slovak raw sheep milk cheeses against 2 foodborne pathogens (indicator strains)

Indicator strain ¹	Diameter of inhibition zone, ² mm			
	L5	L19	L20	L22
<i>Listeria monocytogenes</i> CCM 5676	8.6	8.2	10.1	9.4
<i>Staphylococcus aureus</i> CCM 5756	12.1	9.7	10.4	0

¹CCM = Czech Collection of Microorganisms (Brno, Czech Republic).²Entry of 0 indicates diameter ≤ 8 mm.

differences were observed between WBC and CFNS. Although WBC showed inhibitory activity against both *E. coli* and *Bacillus cereus* reference strains, CNFS from the same cultures did not cause visible inhibition. Therefore, it can be assumed that both *E. coli* and *B. cereus* reference strains were inhibited by low pH values, resulting from lactic acid production by WBC of lactobacilli.

Enterotoxigenic *Staphylococcus aureus* is often detected in raw sheep milk cheeses, including Bryndza cheese (Mašlanková et al., 2009), and causes problems with ready-to-eat foods for immunodeficient individuals and producers of raw milk cheese (Carfora et al., 2015). As observed in this study, 4 wild strains of *Staph. aureus* were effectively inhibited by CFNS of *Lb. plantarum* cultures.

Finally, antimicrobial activity was tested in partially purified bacteriocins (PPB) of *Lb. plantarum* strains L5, L19, L20, and L22 against the cell cultures of *Listeria monocytogenes* and *Staph. aureus* reference strains. As shown in Table 2, antimicrobial activity was confirmed in strains L5 and L19, despite the fact that their CFNS did not inhibit any of the foodborne pathogens tested; this particularity could be an effect of concentration and purification of the bacteriocin peptides in the PPB extract. In contrast, the PPB of strains L20 and L22 showed antimicrobial activity similar to that of their respective CFNS.

Based on the previous results, we concluded that *Lb. plantarum* strains L5, L19, L20, and L22 show sufficient in vitro antibacterial activity against a broad spectrum of foodborne pathogens, including wild strains of *Staph. aureus*. These strains will further be tested in cooperation with the Slovak dairy sector in an attempt to maintain natural cultures of the traditional Slovak dairy products.

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