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BRYNDZA CHEESE OF SLOVAK ORIGIN AS POTENTIAL RESOURCES OF PROBIOTIC BACTERIA

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ABSTRACT

Bryndza cheese includes several predominant lactic acid bacteria. The aim of our study was the antagonistic effect of lactic acid bacteria supernatant isolated from ewes´ cheese bryndza against ten Gram-positive and Gram-negative bacteria. Isolated strains of bacteria were obtained from bryndza which were produced in five different regions of Slovakia. Isolated strains of lactic acid bacteria were identified with mass spectrometry MALDI-TOF MS Biotyper. A total of one hundred and thirty lactic bacteria include *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus hirae*, *Lactobacillus brevis*, *Lactobacillus harbinensis*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus paracasei* ssp. *paracasei*, *Lactobacillus paraplantarum*, *Lactobacillus suebicus*, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis*, and *Pediococcus acidilactici* were tested in this study against Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonnei* CCM 1373, *Yersinia enterocolitica* CCM 5671 and Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Enterococcus faecalis* CCM 4224, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461, *Streptococcus pneumonia* CCM 4501 with agar diffusion method. Lactic acid bacteria showed activity 92% against *Yersinia enterocolitica*, 91% against *Klebsiella pneumoniae*, 88% against *Escherichia coli*, 84% against *Listeria monocytogenes*. The most effective bacteria against Gram-positive and Gram-negative bacteria tested was *Lactobacillus paracasei* ssp. *paracasei*.

Keywords: bryndza; Gram-positive and Gram-negative bacteria; lactic acid bacteria; probiotic effect; mass spectrometry

INTRODUCTION

Bryndza is a traditional Slovak natural white spreadable ripened cheese with Protected geographical indication status **(Commission Regulation (EC) No. 676/2008)**. Bryndza is made from ewes' or a mixture of ewes' and cows' milk and is a rich source of protein, vitamins, and minerals **(Toth et al., 2016)**. Bryndza is a dairy product that naturally contains a broad spectrum of microorganisms that has crucial importance on cheese properties, flavor, and aroma. Microorganisms are present during the whole process of cheese production, they are important in coagulation and ripening **(Andrade et al., 2008; Tilocca et al., 2020)**. Also, beneficial strains help to inhibit the growth of the pathogens and reduce the spoilage of the dairy products **(Arqués et al., 2015)**.

In previous research Gram-positive, Gram-negative bacteria, and yeasts were found and identified in bryndza **(Kačániová et al., 2019)**. The dominant group of bacteria in bryndza was lactic acid bacteria (LAB), mainly *Lactobacillus* species **(Kačániová et al., 2020)**. *Lactococcus, Pediococcus, Enterococcus, Streptococcus* were abundant in bryndza from different Slovak regions also **(Berta et al., 2009; Šaková et al., 2015; Sádecká et al., 2019)**. The probiotic properties of bacteria isolated from bryndza were observed in *Lactobacillus plantarum*, *Enterococcus faecium*, and *Enterococcus faecalis* strains. Researches claimed that these potentially probiotic strains can inhibit the growth of pathogenic bacteria, and some of them can survive in the acidic gastrointestinal environment, which is necessary for reaching the intestine of the host **(Belicová et al., 2011; Belicová et al., 2013)**.

Probiotic bacteria as an important part of intestinal microbiota helps regulate the immune responses, relieve the gastrointestinal tract dysfunction, alleviate the allergies, or lower cholesterol levels **(Dicks and Botes, 2010**; **Plaza-Diaz et al., 2019)**. Moreover, the anti-carcinogenic properties of probiotic bacteria have been described **(Zhong et al., 2014)**.

Our study aimed to evaluate lactic acid bacteria isolated from ewes´ bryndza and select the most active probiotic bacterial strain against pathogens and opportunistic pathogens.

Scientific hypothesis

Bryndza isolates possess probiotic activity. LAB can inhibit antagonistic activity against pathogens.

MATERIAL AND METHODOLOGY

Isolation of lactic acid bacteria

A total of 130 lactic acid bacteria were isolated from Slovak ewes´ bryndza. The bryndza samples were obtained from five producers in Slovakia. Before identification, the lactic acid bacteria colonies were subcultured on 90% of Trypton Soya agar and 10% of Main Rogosa (MRS) agar (Oxoid) at 30 °C for 18 – 24 h microaerobically. One colony of each bacterial isolate was selected for screening. Subsequently, an analysis of the bacteria identification was performed using the MALDI-TOF MS Biotyper.

Bacterial strains for testing

The bacterial strains of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Pseudomonas aeruginosa* CCM 1959, *Yersinia enterocolitica* CCM 5671 and five of Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Micrococcus luteus* CCM 732, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461, *Streptococcus pneumoniae* CCM 4501 were obtained from the Czech collection of microorganisms (Brno).

Antibacterial activity of LAB isolate

The culture of lactic acid bacteria after 24 h of incubation in MRS broth (Oxoid, Basingstoke, UK) was centrifuged at 5500 g for 10 min at 4 °C and 2 mL of the supernatant was used for detection of antibacterial activity.

The well diffusion assay was used. Bacteria were spread on Petri dishes with MRS agar. LAB isolates were added into 6 mm diameter wells were created into the agar. The amounts of LAB and indicator bacteria were the same (100 µl, 10^8 CFU/mL) prepared from the broth culture of bacteria according to the 0.5 McFarland standard. After 48 h incubation at 37 °C in an aerophilically chamber, the inhibition zone diameter was measured for detection of the antagonistic effect of the LAB isolate against pathogenic bacteria.

Statistical analyses

The mean and standard deviation of inhibition zones was calculated for the detection of antagonistic effect against tested Gram-positive and Gram-negative bacteria.

RESULTS AND DISCUSSION

LAB antimicrobial products, such as bacteriocins, are very important in bioconservation of various foods. The diverse use of LAB bacteriocins, individually or as biopreservative combinations, may help to improve food safety, especially of traditional products **(Jamuna and Jeevaratnam, 2004; Mojgani and Amimia, 2007)**.

In our study 130, lactic acid bacteria isolated from ewes´ cheese bryndza (Table 1) were tested for antimicrobial activity and antagonistic effect against pathogenic gram-positive and Gram-negative bacteria.

Table 1 Number of LAB isolates.

Species of LAB	Number of isolates
Enterococcus faecalis	10
Enterococcus faecium	10
Enterococcus hirae	10
Lactobacillus brevis	10
Lactobacillus harbinensis	10
Lactobacillus johnsonii	10
Lactobacillus plantarum	10
Lactobacillus paracasei* ssp. *paracasei	10
Lactobacillus paraplantarum	10
Lactobacillus suebicus	10
Lactococcus lactis* ssp. *lactis	10
Lactococcus lactis	10
Pediococcus acidilactici	10
Total	130

In agar test, lactic acid bacteria isolated from ewes´ cheese bryndza demonstrated different antimicrobial activity with the inhibition the zone ranged from <1 to >5 mm. Altogether, 92% of LAB showed activity against *Yersinia enterocolitica*, 91% against *Klebsiella pneumoniae*, 88% against *Escherichia coli*, 84% against *Listeria monocytogenes*. Antimicrobial activity lower than 84% was observed against *Salmonella enterica* subsp. *enterica, Pseudomonas aeruginosa, Bacillus thuringiensis, Micrococcus luteus, Staphylococcus aureus* subsp. *aureus,* and *Streptococcus pneumoniae* (Table 2). **Belicová et al. (2013)** tested 125 acid resistant presumptive lactobacilli isolated from bryndza against *Listeria monocytogenes* CCM 4699, *Staphylococcus lentus* CCM 3472, *Acinetobacter calcoaceticus* CCM 4503, *Sphingomonas paucimobilis* CCM 3293, and *Salmonella enterica* subsp. *enterica*, serovar Typhimurium.

LAB produces metabolites such as organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoin, carbon dioxide, and bacteriocins that were classified as antimicrobial agents. Moreover, the production of organic acids resulted in low pH, which also inhibits the activity of pathogenic microorganisms **(Ponce et al., 2008; Šušković et al., 2010)**. Antibacterial activity of organic acids and bacteriocins was confirmed against various pathogenic Gram-positive and Gram-negative microorganisms **(Maragkoudakis et al., 2009)**.

A total of 20 LAB isolates showed strong inhibition zones (more than 5 mm) against *P. aeruginosa* and *M. luteus*. Nineteen of LAB isolates showed strong antimicrobial activity against *S. aureus*. Cell suspension of *Lactobacillus plantarum* inhibited *L. monocytogenes* growth **(Ennahar et al., 1998)**. *Lactococcus lactis* reduced levels of *L. monocytogenes* in Cheddar cheese **(Buyong et al., 1998)**. Lactococci developed by **Reviriego et al. (2005)** and **Reviriego et al. (2007)** reduced the number of *L. innocua, L. monocytogenes, S. aureus,* and *E. coli* in cheese **(Rodríguez et al., 2005)**. The intermediate inhibitory effect against Gram-negative and Gram-positive bacteria (1 – 5 mm) is shown in Figure 1. The most effective strains were *Lactobacillus paracasei* ssp. *paracasei* against all tested pathogens. This bacteria exhibited inhibitory activity against all 10 bacteria tested with 2 to 8 isolated were active against particular bacteria.

Table 2 Number of lactic acid bacteria isolates with antimicrobial effect against pathogenic bacteria in mm.

Inhibition zone	EC	KP	SEE	PA	YE	BT	ML	LM	SAA	SP
<1	5	4	10	15	5	15	20	6	11	15
1 – 5	115	118	105	95	120	105	90	110	100	101
>5	10	8	15	20	5	10	20	14	19	14

Note: EC – *Escherichia coli*; KP – *Klebsiella pneumoniae*; SEE – *Salmonella enterica* subsp. *enterica*; PA – *Pseudomonas aeruginosa*; YE – *Yersinia enterocolitica*; BT – *Bacillus thuringiensis*; ML – *Micrococcus luteus*; LM – *Listeria monocytogenes*; SAA – *Staphylococcus aureus* subsp. *aureus*; SP – *Streptococcus pneumoniae*.

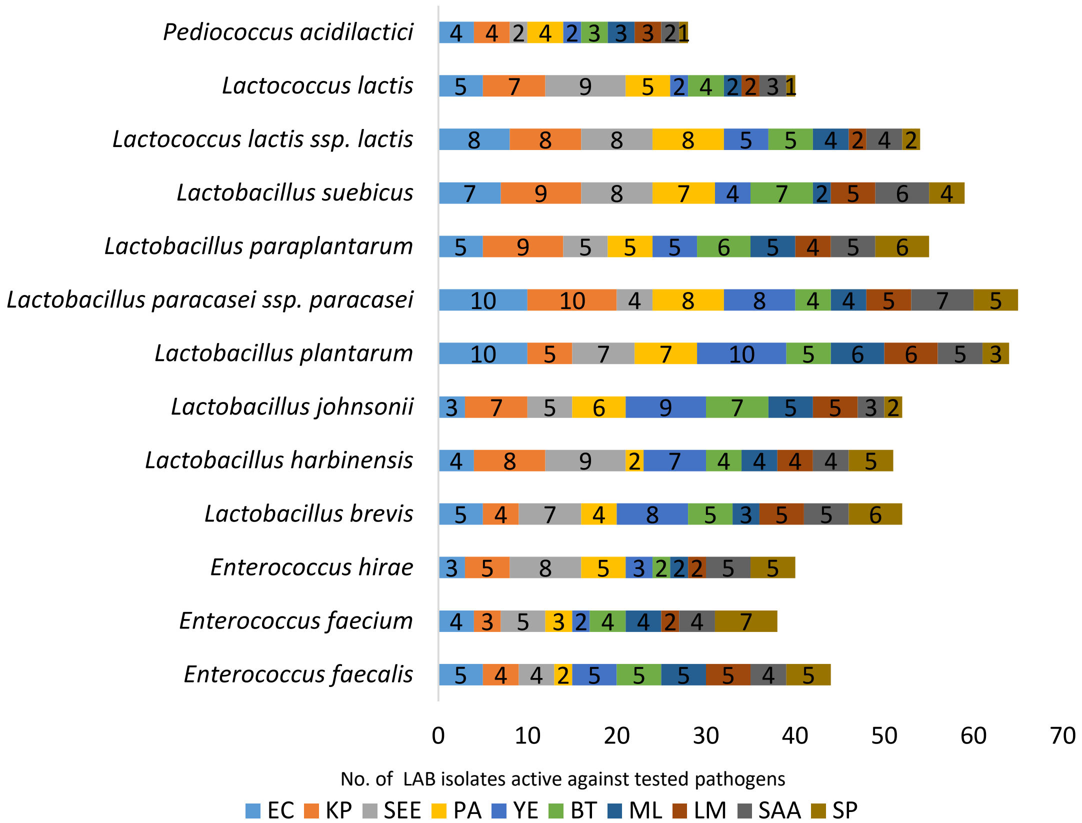

Figure 1 The inhibitory activity of the most active predictive lactic acid isolates against the pathogenic strains. Note: EC – *Escherichia coli*; KP – *Klebsiella pneumoniae*; SEE – *Salmonella enterica* subsp. *enterica*; PA – *Pseudomonas aeruginosa*; YE – *Yersinia enterocolitica*; BT – *Bacillus thuringiensis*; ML – *Micrococcus luteus*; LM – *Listeria monocytogenes*; SAA – *Staphylococcus aureus* subsp. *aureus*; SP – *Streptococcus pneumoniae.*

In total, inhibitory activity was expressed 64 times by 10 *L. paracasei* spp. *paracasei* isolates.

The most effective bacterial strain was *L. lactis* subsp. *lactis* against followed by *L. paraplantarum* against *S. enterica* subsp. *enterica*. The lowest antagonistic effect of lactic acid bacteria was found in *Enterococcus hirae* against *Bacillus thuringiensis* (Table 3).

Foodborne pathogens have become an important social topic and have received much attention from consumers and food safety regulatory agencies around the world because of frequent foodborne outbreaks. In previous studies, LAB showed a wide range of antimicrobial effects against many foodborne pathogens **(Soerjadi et al., 1981; Ennahar and Deschamps, 2000; Messens and De Vuyst, 2002; Dodd, 2012)**. Studies of interaction between LAB and *L. monocytogenes* in various media and with various LAB strains have been performed, and in all cases LAB inhibited the growth of *L. monocytogenes* **(Tharrington and Sorrells, 1992; Zhang et al., 2011; Zhu et al., 2014)**.

However, **Kao and Frazier (1966)** obtained a mixed result when LAB was cocultured with *S. aureus*. Many researchers have found that LAB can inhibit *Salmonella* **(Keersmaecker et al., 2006; Zhang et al., 2016; Yang et al., 2017)** and that the bacteriostatic substances produced by LAB are thermally stable. The research focused on the inhibition of *E. coli* by LAB is more extensive compared to inhibition of the other bacteria. **Du et al. (2016)** found three strains of *Lactobacillus acidophilus* which could inhibit *E. coli* ATCC 25922. Other investigations on *E. coli* O157:H7 proliferation control showed that LAB could effectively inhibit the growth of *E. coli* O157:H7 **(Brashears et al., 1998; Fooladi et al., 2014)**. *Klebsiella pneumonia*, *Bacillus cereus*, *Shigella flexneri* **(Sharma et al., 2017)**, *Staphylococcus epidermidis* **(Diepers et al., 2016)**, and *Candida albicans* **(Yu et al., 2006)** also can be inhibited by LAB.

Table 3 Antagonistic effect of lactic acid bacteria against Gram-negative and Gram-positive bacteria in mm (mean=zone of inhibition of 10 isolates ±*SD*).

LAB	EC	KP	SEE	PA	YE	BT	ML	LM	SAA	SP
Enterococcus faecalis	3.70 ±2.31	3.70 ±1.25	3.30 ±2.06	3.30 ±1.16	3.60 ±1.07	2.40 ±1.07	2.70 ±1.16	2.90 ±0.87	2.50 ±0.53	2.40 ±0.70
Enterococcus faecium	3.90 ±1.73	3.40 ±1.51	3.10 ±2.08	3.40 ±1.00	3.20 ±1.23	2.50 ±0.97	2.60 ±0.97	2.60 ±0.52	2.50 ±1.18	2.50 ±1.08
Enterococcus hirae	3.40 ±1.78	3.20 ±1.40	3.00 ±2.00	3.20 ±1.03	3.00 ±0.82	2.00 ±0.47	2.50 ±0.85	2.70 ±0.68	2.20 ±1.14	2.70 ±1.34
Lactobacillus brevis	4.10 ±1.79	3.80 ±2.10	3.70 ±2.41	3.80 ±2.10	4.40 ±0.97	2.60 ±1.07	2.70 ±0.67	2.90 ±0.74	2.60 ±1.35	3.00 ±1.70
Lactobacillus harbinensis	4.30 ±1.49	4.20 ±1.87	4.70 ±1.06	4.10 ±2.02	4.60 ±0.84	3.00 ±1.15	3.00 ±0.47	3.00 ±0.67	3.00 ±1.41	3.40 ±2.32
Lactobacillus johnsonii	4.60 ±1.27	4.40 ±1.58	4.20 ±2.30	4.40 ±2.07	4.70 ±0.67	2.70 ±1.25	3.10 ±0.32	3.20 ±0.42	3.50 ±1.51	3.30 ±2.54
Lactobacillus plantarum	4.70 ±1.06	5.10 ±1.73	4.50 ±2.01	4.70 ±2.06	4.80 ±0.42	2.90 ±1.10	3.30 ±0.67	3.10 ±0.57	3.60 ±1.35	3.50 ±2.42
Lactobacillus paracasei ssp. *paracasei*	4.90 ±0.88	5.30 ±1.57	5.10 ±2.08	4.90 ±1.85	4.90 ±0.31	3.00 ±1.05	3.60 ±1.07	3.40 ±0.70	3.80 ±1.69	3.50 ±2.84
Lactobacillus paraplantarum	5.00 ±0.67	5.40 ±1.58	5.60 ±1.84	4.80 ±1.81	4.80 ±0.42	3.20 ±1.03	3.70 ±1.06	3.60 ±0.84	3.90 ±1.66	3.70 ±2.87
Lactobacillus suebicus	4.80 ±0.63	5.30 ±1.57	5.00 ±1.76	4.50 ±1.18	4.90 ±0.57	3.30 ±0.95	3.50 ±0.71	3.50 ±0.71	4.00 ±1.56	3.80 ±2.78
Lactococcus lactis ssp. *lactis*	5.10 ±0.74	5.50 ±1.65	5.70 ±1.70	4.70 ±1.25	5.00 ±0.67	3.40 ±0.84	3.60 ±1.35	3.40 ±0.84	3.90 ±1.45	3.80 ±2.62
Lactococcus lactis	4.90 ±0.99	5.30 ±1.57	5.40 ±1.71	4.40 ±1.27	4.80 ±0.92	3.30 ±0.82	3.60 ±1.51	3.20 ±1.14	3.90 ±1.37	3.90 ±2.51
Pediococcus acidilactici	4.40 ±1.51	4.60 ±1.51	4.30 ±1.83	4.10 ±1.20	4.40 ±1.51	3.00 ±1.05	3.00 ±0.94	2.90 ±1.29	3.30 ±1.16	3.20 ±2.20

Note: EC – *Escherichia coli*; KP – *Klebsiella pneumoniae*; SEE – *Salmonella enterica* subsp. *enterica*; PA – *Pseudomonas aeruginosa*; YE – *Yersinia enterocolitica*; BT – *Bacillus thuringiensis*; ML – *Micrococcus luteus*; LM – *Listeria monocytogenes*; SAA – *Staphylococcus aureus* subsp. *aureus*; SP – *Streptococcus pneumoniae*.

CONCLUSION

In conclusion, the present study of lactic acid bacteria strains isolated from ewes´ cheese bryndza confirmed antagonistic effect against Gram-positive and Gram-negative bacteria.

In our study, the best results were found for *Lactococcus lactis* subsp. *lactis* against *Salmonella enterica* subsp. *enterica*. *In vitro* screening of LAB from Slovak bryndza ewes´ cheese have good potential for use as probiotic cultures.

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