Lactic Acid Bacteria Isolated from Traditional Cheese

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Abstract
Different strains of lactic acid bacteria (LAB) could be identified in cheeses made in accordance with national recipes. This strains of LAB are not within the starter cultures used in the dairy industry. The aim of the present study was to identify LAB in traditional cow's milk cheeses of Slovakia. An amount of 40 samples of cheese were produced in the four regions of the Slovakia. LAB were cultured on de Man, Rogosa and Sharpe media for 48 h at 37 °C microaerophilically. The of LAB were confirmed with MALDI-TOF. LAB colonies were subcultured on TSA and 166 cultures were used for further confirmation. The following genera and species were identied with mass spectrometry method: *Lactococcus* spp., including *L. lactis* and *L. garvieae*; *Lactobacillus* spp., including *L. curvatus, L. delbrueckii, L. fermentum, L. papacasei, L. paraplantarum, L. plantarum* and *L. sakei*; *Enterococcus* spp., including *E. durans, E. faecalis, E. faecium* and *E. italicus*; *Leuconostoc* spp., including *L. mesenteroides*; *Pediococcus* spp. with *P. pentosaceus* and *Streptococcus* spp. with *S. salivarius* ssp. *thermophilus*.

Keywords: lactic acid bacteria, MALDI-TOF MS Biotyper, Slovak cheese

1. Introduction

Hundreds of cheese varieties are produced in the world based on the differences both in the type of milk used and in the method of production. However, only a small number of these varieties have commercial importance, and most of them are produced and consumed locally. The production of raw milk cheeses has long been practised in Europe, particularly in France, Italy and Switzerland. Raw milk cheeses are often characterised by richer and stronger flavour intensity than cheeses made from pasteurised milk, and they are considered more natural [1].

The LAB naturally present in raw milk or intentionally added during the cheese manufacturing process are associated with properties such as taste, texture and aroma of dairy products, being largely used as starter cultures in various products of this industry [2]. The LAB group comprehends 16 genera [3], among which the ones more commonly found in cheeses are *Lactococcus, Lactobacillus, Streptococcus, Leuconostoc* and *Enterococcus* [4]. The interest in the microbiota of raw milk cheese and other

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traditional dairy products results from the need of characterization of their complex populations and namely the identification of new strains of LAB) [5]. Traditional dairy products host an enormous pool of microbial genetic diversity, which has a high biotechnological potential and is of great importance to the food industry [6].

The selection of strains as a starter for production of specific fermented products is mostly done with classical microbiological methods. Different parameters of bacterial environments, including the assessment of their morphological, physical, chemical and technological properties was done [7]. New methods applicable in microbiology for microbial systematic involve several techniques, which ae orientated in studying of microbial genome with molecular and genetic approaches. This allow to describe the molecular diversity of microbial species and accumulated the information about their properties [8,9]. New methods allow to type and, hence, select the most suitable strains if the microorganisms, which could be used as a starter culture in food preparation [10]. This strains need to possess the characteristics, which essential for industrial use in food production. The criteria of the safety of their application involves the absence of virulence and susceptibility of antibiotic [11,12]. Another properties of the strains may involve the probiotic abilities by producing bacteriocins and other compounds [7,13], survival in the gastrointestinal tract [11] and decreasing of the level of cholesterol in the blood [14]. The aim of our research was to identify LAB in traditional Slovak cow milk cheese.

2. Materials and methods

2.1. Cheese Samples

The study was conducted in 2018 with 40 non-smoked and smoked parenica cheese were studied. Milk product samples were collected from Bánovce nad Bebravou, Liptovský Mikuláš, Červený Kameň and Važec. Samples were transported to the laboratory with icebox for microbiological investigations. A 5 g of sample was mixed with 45 ml of 0.89 % sterile saline for further preparation of serial dilutions (10^{-2} to 10^{-4}). An amount of 1 ml was plated out on agars.

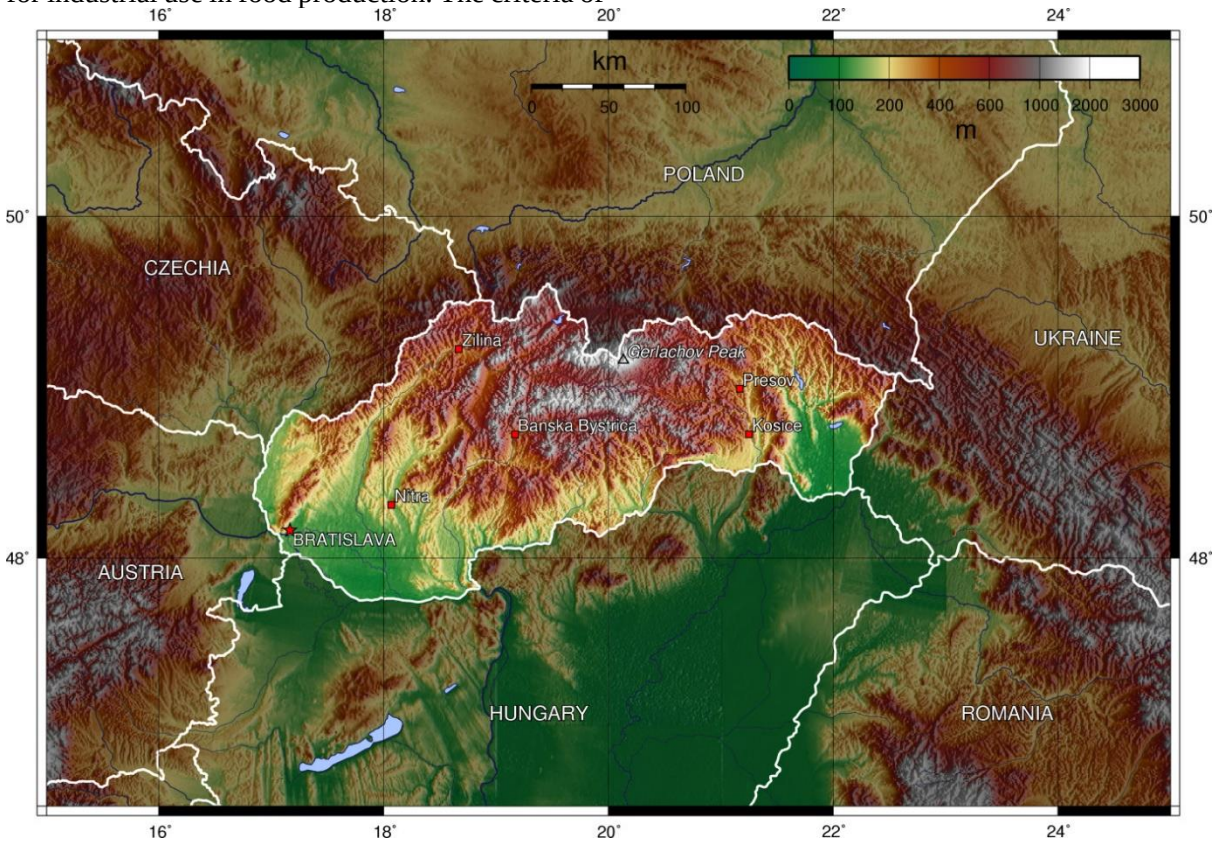

Figure 1. Map of Slovak republic (www.google.sk)

2.2 Isolation of Lactic Acid Bacteria (LAB)

A 1 mL of each dilution was plated out onto MRS agar (de Man, Rogosa and Sharpe, Sigma-Aldrich, St. Louis, USA) and agars were. were incubated for 72 h at 30 °C. Bacterial growth was checked and the colonies with morphology of LAB were confirmed with MALDI-TOF. Selected colonies were cultured overnight at 37 °C on TSA agar (Tryptone Soya Agar) for identification.

2.3 Identification of bacteria with MALDI-TOF MS Biotyper

A sample for MALDI-TOF MS analysis was done in accordance with extraction protocol provided by the manufacturer (Bruker Daltonik, Bremen, Germany). Mass spectra were automatically generated by the microflex LT MALDI-TOF mass spectrometer (Bruker Daltonik, Germany) operated in the linear positive mode within a mass range of 2000-20000 Da. Calibration was done using the Bruker bacterial test standard. Results of mass spectra were processed with the MALDI Biotyper 3.0 software (Bruker Daltonik, Germany). The identification criteria used were: a score of 2.300 to 3.000 - identification on species level; 2.000 to 2.299 - genus identification with probable species identification; 1.700 to 1.999 - identification to the genus level; <1,700 - unreliable identification.

3. Results and discussion

The lactic microbiota of raw milk and traditional dairy products still stimulates interest due to the necessity of identifying the microorganisms responsible for their organoleptic characteristics and, in particular, new strains of LAB, typical of a given product or region [15].

Table 1 shows the family of lactic acid bacteria isolated from cheese, where were found 4 families. From the non-smoked and smoked cheese a total of 17 species of 6 bacterial genera of lactic acid bacteria were identified with MALDI-TOF Mass Spectrometry. From a total of 166 isolates, the representation of each microbial genus reached the following values: 28 isolates of *Enterococcus* spp., 70 isolates of *Lactobacillus* spp. 14 isolates of *Lactococcus lactis*, 14 isolates of *Leuconostoc* spp., 6 isolates of *Pediococcus* spp. and 15 isolates of *Streptococcus* spp.

Table 1. Isolated species of bacteria from traditional Slovak cheese

Family	Genera	Species
Enterococcaceae	*Enterococcus*	*Enterococcus durans*
Enterococcaceae	*Enterococcus*	*Enterococcus faecalis*
Enterococcaceae	*Enterococcus*	*Enterococcus faecium*
Enterococcaceae	*Enterococcus*	*Enterococcus italicus*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus curvatus*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbruckii*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus fermentum*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus paracasei*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus paraplantarum*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus plantarum*
Lactobacillaceae	*Lactobacillus*	*Lactobacillus sakei*
Streptococcaceae	*Lactococcus*	*Lactococcus garvieae*
Streptococcaceae	*Lactococcus*	*Lactococcus lactis*
Leuconostocaceae	*Leuconostoc*	*Leuconostoc mesenteroides*
Lactobacillaceae	*Pediococcus*	*Pediococcus pentosaceus*
Streptococcaceae	*Streptococcus*	*Streptococcus salivarius* ssp. *thermophilus*

Table 2 shows that the most common microorganisms isolated from cheese were *Lactobacillus* spp. The artisanal Coalho cheese produced in Sertão of the State of Paraíba presents a diversified microbiota of LAB, represented by the genera *Enterococcus*, *Lactococcus*, *Streptococcus*, *Lactobacillus*, *Leuconostoc* and *Weissella*. The sequencing of the 16S rRNA gene was a very efficient tool for the identification and differentiation of these microorganisms. The most prevalent species were *Enterococcus faecium*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus garvieae* and *Streptococcus infantarius* susbp. *infantarius*. The distribution of the different LAB species was not the same in all the micro-regions of the Sertão region of the State of Paraíba [15].

A large number of researches indicated that *Lactobacillus* and *Lactococcus* were the most dominant genera in traditional, home-made cheeses [16-18]. Bluma and Ciprovica [19] indicated that the most frequently isolated LAB genera (lactococci and lactobacilli) were found in raw milk. Also, they indicated that identified species were *Lc. lactis*, *Lactobacillus brevis*, and *Lb. fermentum*. Šaková et al. [20] showed high presence of lactococci and lactobacilli in May bryndza cheese. *Lc. Lactis* subsp. *lactis* and *Lc. Lactis* subsp. *Lactis* var. *diacetylactis* were dominant isolates from raw-milk Arzúa-Ulloa cheeses [21], which is in accordance with our research. Giraffa [22] and Strateva et al. [23] noticed that, among enterococci, *E. faecium* and *E. faecalis* were generally the most frequent and prevalent species isolated from cheeses, which was also confirmed in our study.

Table 2. Number of isolates identified with MALDI-TOF MS Biotyper in cheese

Microorganisms	Non- smoked cheese	Smoked cheese	Total	% bacterial species
Enterococcus durans	4	6	10	6..02
Enterococcus faecalis	2	2	4	2.41
Enterococcus faecium	4	5	9	5.42
Enterococcus italicus	2	3	5	3.01
Lactobacillus curvatus	8	6	14	8.43
Lactobacillus delbruckii	5	6	11	6.63
Lactobacillus fermentum	7	6	13	7.83
Lactobacillus paracasei	7	9	16	9.64
Lactobacillus paraplantarum	6	5	11	6.63
Lactobacillus plantarum	5	4	9	5.42
Lactobacillus sakei	5	3	8	4.82
Lactococcus garvieae	5	2	7	4.22
Lactococcus lactis	7	7	14	8.43
Leuconostoc mesenteroides	6	8	14	8.43
Pediococcus pentosaceus	2	4	6	3.61
Streptococcus salivarius ssp. *thermophilus*	7	8	15	9.05
Total	**82**	**84**	**166**	**100**

4. Conclusions

Microbiological analysis of 40 cheese samples revealed the four main groups of lactic acid bacteria: 17 species of 6 bacterial genera of lactic acid bacteria were identified with MALDI-TOF Mass Spectrometry. From a total of 166 isolates, the representation of each microbial genus reached the following values: 28 isolates of *Enterococcus* spp., 70 isolates of *Lactobacillus* spp. 14 isolates of *Lactococcus lactis*, 14 isolates of *Leuconostoc* spp., 6 isolates of *Pediococcus* spp. and 15 isolates of *Streptococcus* spp.

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