Isolation of autochthonous lactic acid bacteria from ewes' lump cheese, bryndza cheese and barrelled ewes' cheese, and their characterization using Fourier transform infrared spectroscopy

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Summary

Autochthonous lactic acid bacteria were isolated from ewes' lump cheese, bryndza cheese and barrelled ewes' cheese produced in Slovakia. The collection of 404 isolates obtained was characterized by Fourier transform infrared (FTIR) spectroscopy. *Lactococcus lactis* subsp. *lactis* was found to be dominant in the majority of samples, followed by *Lactobacillus curvatus*, *Lb. casei* and other *Lactobacillus* spp., *Lactococcus* spp. and *Leuconostoc* spp. FTIR spectroscopy was used also for rapid grouping of *Lactobacillus* colonies grown on de Man - Rogosa - Sharpe agar, as a rapid preparation step prior to molecular-biological identification. Using binary mixtures of selected *Lactobacillus* spp., heterogenity within colonies of one strain on individual plates ranged from 0.082 to 0.338, and heterogenity between two strains ranged from 0.620 to 1.022, which facilitated grouping of colonies of one strain. Results demonstrated that FTIR can be effectively used for characterization of lactic acid bacteria isolated from cheese, including closely related species, and for rapid grouping of isolates at culture-based analysis of biodiversity in cheese.

Keywords

cheese; *Lactobacillus*; *Lactococcus;* identification; Fourier transform infrared spectroscopy; rapid method

Ewes' lump cheese, bryndza cheese and barrelled ewes' cheese are typical Slovakian food products. Since, in a traditional variant of the technology of their production, no microbial starter cultures are used, autochthonous lactic acid bacteria are mostly responsible for the quality of these cheeses. During their ripening, *Lactobacillus* spp., *Lactococcus* spp. and *Leuconostoc* spp. produce important metabolites, such as lactic acid and volatile aroma-active compounds, which contribute to organoleptic characteristics of these food products [1–3].

The progress in molecular biology, DNA sequencing and, recently, in high-throughput sequencing techniques facilitated to obtain detailed information on the composition of microbial consortia in fermented foods, including traditional cheeses. However, it is still often required that microbial strains are isolated, for the purpose of further characterization, development and use in the food processing technology. Culture-based characterization of microbial consortia in cheeses is also known to produce results considerably different from the culture-independent approach [3–6].

At the culture-based approach, various methods are available for identification of colonies of bacteria isolated from cheese. These usually require subculturing, with subsequent phenotypic and/or genotypic characterization. The available methods range from microscopy and biochemical tests to examination based on polymerase chain reaction (PCR), e. g. analysis of randomly amplified polymorphic DNA (RAPD), genus-specific or group-specific analysis, sequencing of a frag-

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ment of 16S rDNA, or even sequencing of the whole genome [4, 7, 8]. Some less laborious, faster and easy-to-perform methods have been also proposed for identification of microorganisms, including lactic acid bacteria. These are matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) [9, 10] and Fourier transform infrared (FTIR) spectroscopy [11, 12]. Identification of microorganisms by both of these methods is based on chemical composition of the cellular material. While, in this application, MALDI-TOF MS fingerprints the highly abundant proteins, it is not known which organic molecules contribute to the microbial fingerprinting region of FTIR spectra [9–12].

In this study, we evaluated FTIR spectroscopy, which is known as a rapid method, requiring a minimum of manual work and no chemicals. However, there are also certain disadvantages of the method, such as requirement for advanced mathematical processing because the obtained spectra contain overlapping bands due to the complexity and heterogenity of the sample. There are also certain practical problems with FTIR instrumentation, such as sensitivity of the electronics, which requires that the instrument is switched on without interruption, or sensitivity of the optics to air humidity, which requires frequent changes of desiccation cartridges. Probably the greatest disadvantage of FTIR spectroscopy in microbiological applications is its low robustness in terms of sensitivity to culture conditions of microorganisms, as the chemical composition of microbial cells is strongly influenced by them. When FTIR is used for identification of bacteria to or below the species level, culture conditions have to be strictly controlled with extreme care devoted to identical temperature, aeration and composition of the media, including the use of powdered media from the same batch. Even then, the discrimination of FTIR is much worse than that of sequencing, either directed to 16S rDNA or other genes, or whole genome sequencing [12–14].

While FTIR spectroscopy apparently cannot compete with molecular-biological methods at high-discrimination identification of lactic acid bacteria, another useful application can be found for it. When isolating *Lactobacillus* spp. on common elective media, such as de Man - Rogosa - Sharpe (MRS) agar, a problem with selection of appropriate colonies for further processing arises due to considerable similarities in colony morphology. Since loss of diversity has to be prevented, many (20–30) colonies are usually picked from each plate and identified by DNA sequencing. This approach often leads to a large amount of redundant data, as a great portion of the colonies usually belongs to the same strain or species. In order to reduce the extent of DNA sequencing, utilization of FTIR to group the colonies would be useful [15, 16].

The aim of this study was to isolate the autochthonous lactic acid bacteria from ewes' lump cheese, bryndza cheese and barrelled ewes' cheese, and to characterize them using FTIR spectroscopy. Further aim was to test FTIR spectroscopy at rapid grouping of *Lactobacillus* colonies grown on MRS agar, regarding its robustness and discriminative power in practical microbiological setting, i.e. in loosely controlled conditions. A challenge of this approach is that, unlike bacterial cultures grown in shaken liquid media, colonies may contain cells of different chemical composition, depending on the place in the colony where the cell is located, reflecting different access to air and nutrients. Individual colonies may also differ, depending on the position of the colony, proximity of other colonies of the same or other strain, reflecting different access to nutrients or exposition to metabolites. For this purpose, binary mixtures of selected *Lactobacillus* spp. were prepared, cultured on MRS agar plates and analysed by FTIR spectroscopy.

MATERIALS AND METHODS

Reference bacterial strains

The following strains, *Lactobacillus acidophilus* CCM 4833, *Lb. brevis* CCM 1815, *Lb. casei* CCM 4791, *Lb. curvatus* CCM 7271, *Lb. delbrueckii* CCM 7191, *Lb. fermentum* CCM 7272, *Lb. helveticus* CCM 4280, *Lb. paracasei* CCM 1752, *Lb. pentosus* CCM 4619, *Lb. plantarum* CCM 1904, *Lb. rhamnosus* CCM 7091, *Lactococcus lactis* subsp. *cremoris* CCM 2106, *Lc. lactis* subsp. *lactis* CCM 1877, *Leuconostoc mesenteroides* subsp. *dextranicum* CCM 2085 and *Leu. mesenteroides* subsp. *cremoris* CCM 2078 were obtained from Czech Collection of Microorganisms, Brno, Czech Republic.

Cheese samples

Cheese samples were obtained during years 2012 and 2013 from small and medium-sized ewes' milk processing factories in Central Slovakia. Samples of ewes' lump cheese were obtained from 3 factories, samples of bryndza cheese were obtained from 7 factories and samples of barrelled ewes' cheese were obtained from various barrels from one factory. The samples were transported to the laboratory at 5 °C and isolation of lactic acid bacteria from them was started within 24 h.

Isolation of lactic acid bacteria

A portion of 10 g from a cheese sample was mixed with 90 ml of buffered peptone water (BPW; Merck, Darmstadt, Germany) and homogenized in Stomacher 400 (Seward, Basingstoke, United Kingdom) for 1 min at medium intensity. The homogenate was decimally diluted with BPW, 0.2 ml of the dilution 10^{-5}, 10^{-6} and 10^{-7} was streaked on de Man - Rogosa - Sharpe (MRS) agar (Biokar, Beauvais, France) and the plates were incubated microaerobically in an anaerostat using Anaerocult A (Merck) at 37 °C for 72 h. Additionally, 0.2 ml of the given dilutions were streaked on M17 agar (Biokar) and cultured aerobically at 30 °C for 72 h.

Preparation of single-strain samples for FTIR spectroscopy

Lactic acid bacteria were cultured in MRS medium (Merck) for 16 h at 37 °C with slow shaking of 2 Hz. The cultures were decimally diluted with 0.9% NaCl and, from the dilution 10^{-3}, 10^{-4} and 10^{-5}, a volume of 0.2 ml of the mixture was streaked on MRS agar. Plates were cultured microaerobically for 72 h at 37 °C. From the plates with clearly separated colonies, 2–3 loopfuls (loop volume 1 μl) of the cellular material were taken and suspended in 100 μl of distilled water. The suspension containing whole cells was thoroughly mixed by vortexing for 1 min and a volume of 35 μl of it was pipetted on a position on a 96-position ZnSe measuring plate for FTIR spectroscopy. The plate with applied samples was dried at 37 °C for 45 min and immediately measured by FTIR spectroscopy.

Preparation of binary mixed samples for FTIR spectroscopy

Lactobacillus spp. were cultured in pure cultures in MRS medium for 16 h at 37 °C with slow shaking of 2 Hz. The cultures were decimally diluted with 0.9% NaCl and binary mixtures were prepared from the dilution 10^{-4}. A volume of 0.2 ml of the mixture was streaked on MRS agar and the plates were cultured microaerobically for 72 h at 37 °C. Six plus six bacterial colonies, out of 100–130 colonies grown on a plate, were picked directly from the plate by an inoculation loop, based on visual evaluation of colony morphology, and each was suspended in 100 μl of distilled water. The suspension containing whole cells was mixed by vortexing for 1 min and 35 μl of the suspension was pipetted on a position on a 96-position Zn-Se plate. The plate was dried for 45 min at 37 °C and immediately measured by FTIR spectroscopy.

FTIR spectroscopy

A Bruker Tensor 27 FTIR spectrometer equipped with HTS-XT module (Bruker Optics, Ettlingen, Germany) was used. The spectra were recorded in a range from 4000 cm^{-1} to 400 cm^{-1}, with spectral resolution of 4 cm^{-1} and 32 scans per sample. The spectra were processed by OPUS software (Bruker), which involved averaging of 32 scans, calculation of first derivative by Savitzky-Golay algorithm with 9 smoothing points, and vector-normalization in the region from 1780 cm^{-1} to 720 cm^{-1}. Cluster analysis was carried out by OPUS software on the basis of Euclidean distances using Ward's algorithm, heterogenities being assigned values from 0 to 2.

RESULTS AND DISCUSSION

From ewes' lump cheese, bryndza cheese and barrelled ewes' cheese samples, lactic acid bacteria were cultured on elective microbiological media. The colonies on MRS and M17 agar media were visually inspected for their shape, diameter, surface, edge, consistency and colour. On the basis of characteristic colony morphology, 404 isolates were selected that were presumed to be *Lactobacillus* spp. or *Lactococcus* spp. Individual isolates were subcultured in liquid MRS and on MRS agar, and then subjected to FTIR spectroscopy. The practical protocol of the sample preparation for FTIR spectroscopy was optimized regarding the density and volume of the suspension applied to the measurement plate, so as to obtain good quality spectra as evaluated by the spectrometer's internal software. An illustrative picture of a measurement plate with the applied suspensions of bacterial cells visible as spots of an opaque film inside the circles is given in Fig. 1. Illustrative FTIR spectra of two *Lacobacillus* spp. are given in Fig. 2. The FTIR spectra of the unknown bacterial isolates were compared with the internal database of FTIR spectra, which was prepared using reference strains.

In ewes' lump cheeses from Factory 1, the highest similarity was determined for Isolate 12, spectrum of which showed 99.9% similarity with that of *Lb. casei*, and the lowest similarity was determined for Isolate 124, spectrum of which showed 82.3% similarity with that of *Lb. paracasei.* In Factory 2, the highest similarity was determined for Isolate 272, spectrum of which showed 99.6% similarity with that of *Lb. brevis*, and the lowest similarity was determined for Isolate 263, spectrum of which showed 90.5% similarity with that of *Lc. lactis* subsp. *cremoris.* In Factory 3, the highest

similarity was determined for Isolate 357, spectrum of which showed 99.9% similarity with that of *Lb. paracasei*, and the lowest similarity was determined for Isolate 310, spectrum of which showed 85.0% similarity with that of *Lc. lactis* subsp. *lactis.* Among all 225 isolates from ewes' lump cheeses, the highest prevalence was determined for *Lc. lactis* subsp. *lactis* (132 isolates), followed by *Lb. curvatus* (35 isolates), *Lb. rhamnosus* (20 isolates), *Lb. brevis* (14 isolates), *Lb. casei* (9 isolates), *Lc. lactis* subsp. *cremoris* (8 isolates), *Lb. paracasei* (6 isolates) and *Lb. pentosus* (1 isolate). Spectra of 25 isolates had similarities with the internal database above 99%, while spectra of 143 isolates had a similarity between 97% and 99%, spectra of 41 isolates had a similarity between 95% and 97%, and spectra of 16 isolates (approx. 7% from the total number of isolates) had a similarity lower than 95%.

In bryndza cheese, the highest similarity was determined for Isolate 335, spectrum of which showed 99.9% similarity with that of *Lb. plantarum*, and the lowest similarity was determined for Isolate 306, spectrum of which showed 94.1% similarity with that of *Leu. mesenteroides* subsp. *cremoris.* Among all 53 isolates from bryndza cheese, the highest prevalence was determined for *Lc. lactis* subsp. *lactis* (27 isolates), followed by *Lb. casei* (6 isolates), *Lb. curvatus*, *Lb. paracasei*, *Lb. rhamnosus* and *Lb. brevis* (3 isolates of each species), *Leu. mesenteroides* subsp. *cremoris* (2 isolates) and *Lc. lactis* subsp. *cremoris* (1 isolate). Spectra of 12 isolates had similarities with the internal database above 99%, while spectra of 33 isolates had a similarity between 97% and 99%, spectra of 6 isolates had a similarity between 95% and 97%, and spectra of 2 isolates (less than 4% from the total number of isolates) had a similarity lower than 95%.

In barrelled ewes' cheese, the highest similarity was determined for Isolate 305, spectrum of which showed 99.5% similarity with that of *Lc. lactis* subsp. *lactis*, and the lowest similarity was determined for Isolate 381, spectrum of which showed 88.7% similarity with that of *Lb. rhamnosus.* Among all 36 isolates from barrelled ewes' cheese, the highest prevalence was determined for *Lc. lactis* subsp. *lactis* (13 isolates), followed by *Lb. curvatus* (6 isolates), *Leu. mesenteroides* subsp. *cremoris* (5 isolates), *Leu. mesenteroides* subsp. *dextranicum* (4 isolates), *Lb. brevis*, *Lb. pentosus* and *Lb. casei* (2 isolates of each species), and *Lb. rhamnosus* and *Lc. lactis* subsp. *cremoris* (1 isolate of each species). Spectra of 4 isolates had similarities with the internal database above 99%, while spectra of 15 isolates had a similarity between 97% and 99%,

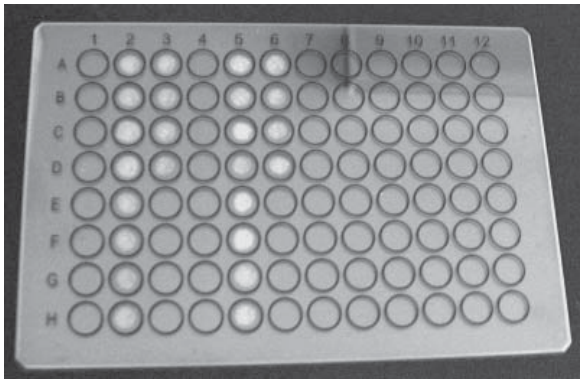

Fig. 1. Zn-Se measurement plate with applied and dried suspensions of *Lactobacillus* strains.

6 replicates were measured. Position: A2–F2 – *Lb. rhamnosus*, G2–D3 – *Lb. pentosus*, A5–F5 – *Lb. acidophilus*, G5–D6 – *Lb. helveticus*.

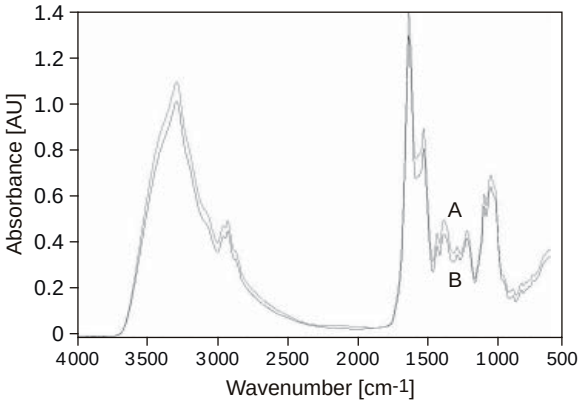

Fig. 2. FTIR spectrum of *Lb. acidophilus* and *Lb. rhamnosus*.

A – *Lb. acidophilus* (upper line), B – *Lb. rhamnosus* (lower line).

spectra of 8 isolates had a similarity between 95% and 97%, and spectra of 9 isolates (25% from the total number of isolates) had a similarity lower than 95%.

The identification results obtained in this study by FTIR spectroscopy are in overall agreement with previous data on the microflora of Slovakian ewes' lump cheese, bryndza cheese and barrelled ewes' cheese obtained by culture-based methods coupled to molecular-biological identification and by culture-independent methods [3, 17–19]. The data are also, to a certain extent, compatible with data from studies on other ewes' cheeses [20–22]. FTIR spectroscopy proved to provide interesting results, although the identification of lactic acid bacteria by this method was less reliable than by molecular-biological methods, as can be seen from similarity values. These parameters could be im-

Tab. 1. Discrimination (in terms of heterogenity) of FTIR spectroscopy between colonies of *Lactobacillus* spp.

		Partner species B								
		Lb. helveticus			*Lb. acidophilus*			*Lb. pentosus*		
Heterogenity		within A	within B	A vs B	within A	within B	A vs B	within A	within B	A vs B
Partner species A	*Lb. rhamnosus*	0.103	0.097	1.022	0.192	0.150	0.851	0.082	0.083	0.658
	Lb. pentosus	0.292	0.338	0.749	0.259	0.123	0.620			
	Lb. acidophilus	0.290	0.124	0.797						

proved by adding more species and strains in the database and/or by a tighter control of culture conditions. However, the latter would compromise the main advantages of the method, i.e. speed and practical simplicity.

In this study, we also tested FTIR spectroscopy at the application to rapid grouping of *Lactobacillus* colonies grown on MRS agar, as a preparation step prior to molecular-biological identification. For this purpose, binary mixtures of 4 selected *Lactobacillus* spp. were prepared, cultured on MRS agar and analysed by FTIR spectroscopy. Results on discrimination, in terms of heterogenity, of FTIR spectroscopy for all strain combinations are given in Tab. 1. An example of a dendrogram, as a result of cluster analysis of data from one plate, is given in Fig. 3. On individual plates, considerable variation within FTIR spectra for colonies of one strain was observed,

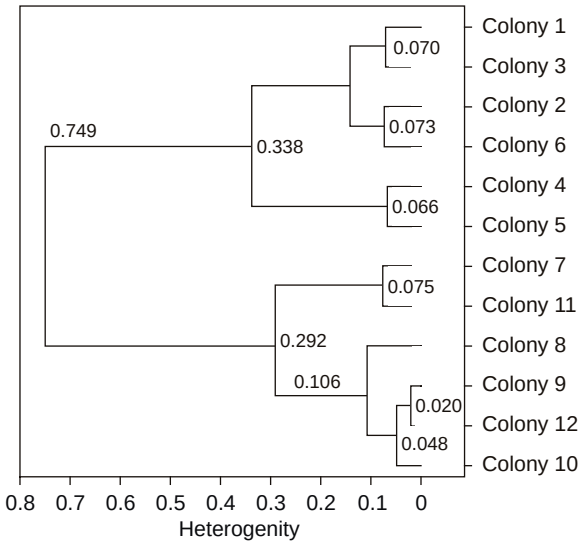

Fig. 3. Dendrogram of discrimination between *Lb. helveticus* and *Lb. pentosus* colonies by FTIR spectroscopy.

Colonies 1–6 were *Lb. helveticus*, colonies 7–12 were *Lb. pentosus*, based on comparison with the internal database of FTIR spectra.

but heterogenity did not exceed the value of 0.338 (this was in case of *Lb. helveticus* when combined with *Lb. pentosus*). The heterogenity values for colonies of one strain were markedly lower than the heterogenity values between the two strains at all combinations, which ranged from 0.620 to 1.022.

The results suggest that the proposed method is suitable for grouping colonies of *Lactobacillus* spp. The method is comparatively rapid and simple, with the hands-on time of approx. 3 min per sample, though the entire measurement was prolonged due to the time of drying of the 96-place measurement plate during 45 min. The FTIR spectroscopic measurement took approx. 1 min per sample, and processing of the spectra further approx. 12 min for the entire plate (12 spectra). The method can be used for grouping of colonies from a plate at culture-based analysis of lactic acid bacterial consortia e.g. in cheese. Using the set heterogenity threshold, one representative per group is selected and then subcultured and identified by DNA sequencing. This will avoid extensive sequencing of identical strains and production of redundant data. The heterogenity threshold may be adapted on the basis of the intermediate sequencing results for specific microbial consortia and matrices. The limitations of the method may be in the colony size, as the cellular material from one colony should be sufficient for measurement of a good quality FTIR spectrum. This may restrict the application of the approach to microorganisms that form larger colonies on the elective agar medium, thus excluding lactococci, pediococci or *Leuconostoc* spp. grown on MRS agar.

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REFERENCES

1. Görner, F.: Der Brinsenkäse aus Schafmilch (Brimsen). Nahrung, *4*, 1980, pp. 157–162.
2. Görner, F. – Valík, Ľ.: Mikrobiológia syrov. In: Görner, F. – Valík, Ľ. (Eds.): Aplikovaná mikrobiológia požívatín. Bratislava : Malé centrum, 2004, pp. 273–338. ISBN 80-967064-9-7.
3. Pangallo, D. – Šaková, N. – Koreňová, J. – Puškárová, A. – Kraková, L. – Valík, L. – Kuchta, T.: Microbial diversity and dynamics during the production of May bryndza cheese. International Journal of Food Microbiology, *170*, 2014, pp. 38–43. DOI: 10.1016/j.ijfoodmicro.2013.10.015.
4. Coeuret, V. – Dubernet, S. – Bernardeau, M. – Gueguen, M. – Vernoux, J. P.: Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Lait, *83*, 2003, pp. 269–306. DOI: 10.1051/lait:2003019.
5. Quigley, L. – O'Sullivan, O. – Beresford, T. P. – Ross, R. P. – Fitzgerald, G. F. – Cotter, P. D.: High-throughput sequencing for detection of subpopulations of bacteria not previously associated with artisanal cheeses. Applied and Environmental Microbiology, *78*, 2012, pp. 5717–5723. DOI: 10.1128/AEM.00918-12.
6. Cocolin, L. – Alessandria, V. – Dolci, P. – Gorra, R. – Rantsiou, K.: Culture independent methods to assess the diversity and dynamics of microbiota during fermentation. International Journal of Food Microbiology, *167*, 2013, pp. 29–43. DOI: 10.1016/j.ijfoodmicro.2013.05.008.
7. Temmerman, R. – Huys, G. – Swings, J.: Identification of lactic acid bacteria: culture-dependent and culture-independent methods. Trends in Food Science and Technology, *15*, 2004, pp. 348–359. DOI: 10.1016/j.tifs.2003.12.007.
8. Pogačić, T. – Kagkli, D. M. – Sikora, S. – Kalit, S. – Havranek, J. – Samaržija, D.: Experimental approaches for identification of indigenous lactococci isolated from traditional dairy products. Mljekarstvo, *61*, 2011, pp. 3–14.
9. Angelakis, E. – Million, M. – Henry, M. – Raoult, D.: Rapid and accurate bacterial identification in probiotics and yoghurts by MALDI-TOF mass spectrometry. Journal of Food Science, *76*, 2011, pp. M568–M572. DOI: 10.1111/j.1750-3841.2011.02369.x.
10. Dingle, T. C. – Butler-Wu, S. M.: MALDI-TOF mass spectrometry for microorganism identification. Clinics in Laboratory Medicine, *33*, 2013, pp. 589–609. DOI: 10.1016/j.cll.2013.03.001.
11. Curk, M. C. – Peledan, F. – Huber, J. C.: Fourier transform infrared (FTIR) spectroscopy for identifying *Lactobacillus* species. FEMS Microbiology Letters, *123*, 1994, pp. 241–248. DOI: 10.1111/j.1574-6968.1994.tb07231.x.
12. Wenning, M. – Scherer, S.: Identification of microorganisms by FTIR spectroscopy: perspectives and limitations of the method. Applied Microbiology and Biotechnology, *97*, 2013, pp. 7111–7120. DOI: 10.1007/s00253-013-5087-3.
13. Oust, A. – Moretro, T. – Kirschner, C. – Narvhus, J. A. – Kohler, A.: FT-IR spectroscopy for identification of closely related lactobacilli. Journal of Microbiological Methods, *59*, 2004, pp. 149–162. DOI: 10.1016/j.mimet.2004.06.011.
14. Dec, M. – Urban-Chmiel, R. – Gnat, S. – Puchalski, A. – Wernicki, A.: Identification of *Lactobacillus* strains of goose origin using MALDI-TOF mass spectrometry and 16S-23S rDNA intergenic spacer PCR analysis. Research in Microbiology, *165*, 2014, pp. 190–201. DOI: 10.1016/j.resmic.2014.02.003.
15. Tindall, B. J. – Brambilla, E. – Steffen, M. – Neumann, R. – Pukall, R. – Kroppenstedt, R. M. – Stackebrandt, E.: Cultivatable microbial diversity: gnawing at the Gordian knot. Environmental Microbiology, *2*, 2001, pp. 310–318. DOI: 10.1046/j.1462-2920.2000.00108.x.
16. Chebeňová, V. – Berta, G. – Kuchta, T. – Brežná, B. – Pangallo, D.: Randomly-amplified microsatellite polymorphism for preliminary typing of lactic acid bacteria from bryndza cheese. Folia Microbiologica, *55*, 2010, pp. 598–602. DOI: 10.1007/s12223-010-0096-4.
17. Berta, G. – Chebeňová, V. – Brežná, B. – Pangallo, D. – Valík, Ľ. – Kuchta, T.: Identification of lactic acid bacteria in Slovakian bryndza cheese. Journal of Food and Nutrition Research, *48*, 2009, pp. 65–71. <http://www.vup.sk/download.php?bulID=97>
18. Chebeňová-Turcovská, V. – Ženišová, K. – Kuchta, T. – Pangallo, D. – Brežná, B.: Culture-independent detection of microorganisms in traditional Slovakian bryndza cheese. International Journal of Food Microbiology, *150*, 2011, pp. 75–78. DOI: 10.1016/1.ijfoodmicro.2011.07.020.
19. Šaková, N. – Sádecká, J. – Lejková, J. – Puškárová, A. – Koreňová, J. – Kolek, E. – Valík, Ľ. – Kuchta, T. – Pangallo, D.: Characterization of May bryndza cheese from various regions in Slovakia based on microbiological, molecular and principal volatile odorants examination. Journal of Food and Nutrition Research, *54*, 2015, pp. 239–251. <http://www.vup.sk/download.php?bulID=1657>
20. Comunian, R. – Paba, A. – Daga, E. S. – Dupré, I. – Scintu, M. F.: Traditional and innovative production methods of Fiore Sardo cheese: a comparison of microflora with a PCR-culture technique. International Journal of Dairy Technology, *63*, 2010, pp. 224–233. DOI: 10.1111/j.1471-0307.2010.00581.x.
21. Alegría, Á. – Szczesny, P. – Mayo, B. – Bardowski, J. – Kowalczyk, M.: Biodiversity in oscypek, a traditional Polish cheese, determined by culture-dependent and -independent approaches. Applied and Environmental Microbiology, *78*, 2012, pp. 1890–1898. DOI: 10.1128/AEM.06081-11.
22. Feutry, F. – Oneca, M. – Berthier, F. – Torre, P.: Biodiversity and growth dynamics of lactic acid bacteria in artisanal PDO Ossau-Iraty cheeses made from raw ewe's milk with different starters. Food Microbiology, *29*, 2012, pp. 33–42. DOI: 10.1016/j.fm.2011.08.011.

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