Occurrence of abortive infection systems and phage resistance in lactic acid bacteria isolated from bryndza ewes' cheese

KATARÍNA DUBÍKOVÁ – PETER PRISTAŠ – PETER JAVORSKÝ

Summary
Sixty-eight bacterial isolates of lactococci, lactobacili and enterococci obtained from bryndza ewes' cheese were identified using Matrix-assisted laser desorption ionization-time of flight (MALDI TOF) analysis of protein spectra. All lactococcal isolates from bryndza were identified as *Lactococcus lactis*. *Lactobacillus plantarum* was found to be a dominant species among lactobacilli, followed by *Lb. paracasei*. Enterococcal population of bryndza cheese consisted mainly of *Enterobacter faecium*, followed by *E. durans* and *E. faecalis*. The presence of genes encoding for 23 abortive infection systems (Abi) in the isolates was tested by polymerase chain reaction analysis. Eleven out of 24 *Lc. lactis* isolates possessed AbiB system. No Abi system was detected in *Lactobacillus* spp. AbiC, AbiD, AbiEi and AbiI were found in enterococcal isolates. In parallel experiments, all isolates were tested for sensitivity to bacteriophage infection. No clear correlation was found between the presence of abortive infection systems and resistance against bacteriophages.

Keywords
lactic acid bacteria; raw milk; phage defence systems; bacteriophage

Lactic acid bacteria (LAB) are a part of starter cultures which are often used in the industry for the production of fermented foods. Starter cultures have crucial roles to play during all phases of the cheese making. Cheese production is based on LAB ability to ferment saccharides, in particular glucose and galactose, so to produce lactic acid and aroma substances that give typical flavour and taste to cheeses. Because approximately 10^{14} bacterial cells are needed for the production of 1 t of cheese, LAB play an important role in the production of artisanal cheeses. The microbial population inhabiting artisanal cheeses is very diverse, where lactococci, lactobacilli and enterococci represent an essential part [1]. In the non-sterile environment of raw or heat-treated milk, these bacteria are sensitive to the presence bacteriophages often found in milk, and may seriously impact the production of milk-based foods, particularly as the fermentation failure [2].

Bacteriophage infection leads to the lysis of a large number of bacterial cells, or even stopping the lactic acid production. The consequence is the poor quality of the products. In the worst case, the inoculated milk must be discarded. For decades, this problem has been studied in the dairy industry, its economic impact stimulated research and bacteriophages of lactic acid bacteria now belong to the best-studied phage systems. Bacteria possess variety mechanisms to prevent bacteriophage infection. These "anti-phage systems" were divided into 5 groups: systems that inhibit phage adsorption systems that block phage infection, phage abortive infection systems; clustered regularly interspaced short palindromic repeats (CRISPR) systems and restriction-modification systems [3].

Phage abortive infection (Abi) systems, also called phage exclusion systems, operate in a counter-intuitive fashion, causing the virus-infected bacterial cell to commit suicide. This can be con-

Katarína Dubíková, Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia; Department of Biochemistry, Faculty of Natural Science, P. J. Šafárik University, Moyzesova 11, 040 01, Košice, Slovakia.
Peter Pristaš, Peter Javorský, Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia.

Correspondence author:
Peter Pristaš, e-mail: pristas@saske.sk

sidered an altruistic act to protect the clonal population. This decreases the number of bacteriophage progeny particles and limits their spread to other cells allowing the bacterial population to survive. Most Abi systems were identified on plasmids of Gram-positive lactococci [4–8] used in dairy fermentations, but lot of them remain incompletely understood regarding their action mechanism and ground of bacterial cell death. It is clear that these mechanisms are varied and can rely upon two or more partners [9]. Twenty-two Abi systems, mainly from different *Lactococcus lactis* strains, have been characterized at nucleotide level. Almost all of lactococcal Abi systems are plasmid-encoded, except for two systems, AbiH and AbiN, which are chromosomally-encoded [10]. Only limited data are available on the occurrence and frequency of Abi systems in LAB.

The main aims of this study were to detect the presence of the genes encoding for abortive infection systems in strains of *Lactococcus*, *Lactobacillus* and *Enterococcus* genera, isolated from bryndza ewes' cheese, and to evaluate their role in protection against bacteriophage infections.

MATERIALS AND METHODS

Isolation and identification of bacterial isolates

All bacterial isolates used in this study were collected by the Dairy Research Institute (Žilina, Slovakia). A sample of 1 g of bryndza cheese was taken and transferred to a separate sterile plastic tube. Each sample was homogenized in 9 ml of sterile saline solution (0.8% NaCl, pH 7). Five 5-fold dilutions of the homogenates were prepared and inoculated on de Man-Rogosa-Sharpe (MRS), M17 and Todd-Hewitt (TH) agar plates (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA) and incubated aerobically for 36 h at 32 °C. Colonies with typical characteristics were randomly selected from plates, tested by Gram staining and their cell morphology was inspected.

The selected isolates were identified by analysis of protein profiles by Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectroscopy (MS) using Maldi Biotyper instrument (Bruker Daltonics, Bremen, Germany). Each sample was spotted on the target plate, overlaid with 1 μl of matrix solution containing the matrix for MALDI-TOF MS, 1.5 mg of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile with 2.5% trifluoro acetic acid, and air-dried.

Measurements were performed with Microflex mass spectrometer (Bruker Daltonics) using FlexControl software (version 3.0, Bruker Daltonics). For each spectrum, 240 shots in 40-shot steps from different positions of the target spot (automatic mode) were collected and analysed. The spectra were imported to BioTyper software (version 2.0; Bruker Daltonics) and were analysed by standard pattern matching with default settings. Results of the pattern-matching process were expressed as proposed by the manufacturer with scores ranging from 0 to 3. For each isolate, the highest score of a match against a spectrum in the database was used for identification [11]. MALDI-TOF protein spectroscopy is a modern, powerful, rapid, precise and cost-effective method for identification of bacteria, compared to conventional phenotypic techniques or molecular techniques based on 16S rRNA analysis [11].

Bacteriophage used

All LAB isolates from bryndza cheese were tested for sensitivity to infections.

Lactobacilli were tested for sensitivity to *Lactobacillus plantarum* phage 8014-B1 (ATCC No. 8014-B1). The bacteriophage was propagated in *Lb. plantarum* strain 175 as a host, grown at 37 °C in MRS broth.

Lactococci were tested for sensitivity to *Lactococcus lactis* phage P008 (DSM No. 10567). The bacteriophage was propagated in *Lc. lactis* subsp. *lactis* DSM 4366 strain in M17 medium at 37 °C.

Enterococci were tested for sensitivity to MSF1 bacteriophage [12]. The bacteriophage was propagated in strain *E. faecalis* 47/3 as a host, in Todd-Hewitt medium at 37 °C.

Bacteriophage suspensions were prepared by a standard procedure [13].

Double agar layer bacteriophage sensitivity assay

Sensitivity to bacteriophages was tested using the double agar layer method according to ADAMS [14]. Small volume (10 μl) of phage suspension (of about 10^7 plaque forming units, PFU) and small volume (50 μl) of host cells grown to a high cell density (of about 10^7 colony forming units, CFU) were mixed in 3 ml of molten 0.6% soft agar medium at 46 °C (multiplicity of infection 1:1). The resulting suspension was poured over a 1% agar to form a thin layer, which hardened and immobilized the bacteria. The bacteriophage infection led to the appearance of visible, circular area of clearing in the confluent bacterial growth known as a plaque. Plaques were scored after 24 h incubation at 37 °C. According to the presence or absence of plaques, the strains were differentiated into two categories: sensitive (with plaque present) or resistant (no plaque present).

Genomic DNA isolation

For preparation of genomic DNA of all strains, GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, Missouri, USA) was used with several modifications. An overnight culture (3 ml) was centrifuged at 10000 ×*g* (microcentrifuge 5415R; Eppendorf, Brinkmann Instruments, Westbury, New York, USA) at 25 °C for 2 min and resuspended in 500 μl of 50 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA). A volume of 100 μl of a 35 mg·ml^{-1} solution of lysozyme (L6876; Sigma, Milwaukee, Wisconsin, USA) was added to the cell suspension and incubated for 1 h at 37 °C with intermittent shaking. The suspension was centrifuged (10000 ×*g* at 25 °C for 1 min) and the sediment gently resuspended in 650 μl of nuclei lysis solution C (Sigma-Aldrich Chimie, Lyon, France). After thorough resuspension, 200–300 μl of ethanol (95–100%) was added to the mixture and vortexed at medium speed for 5 s. The lysate was centrifuged at 10000 ×*g* at 25 °C for 1 min, and the supernatant fluid transferred into the binding column. After a centrifugation step (10000 ×*g* for 1.5 min), supernatant was removed and the column was washed two times by wash solutions according to the protocol (Sigma-Aldrich Chimie). After washing, DNA was eluted by 100 μl of the Elution Solution (Sigma-Aldrich Chimie). Preparations were analysed by electrophoresis (100 V for 20–40 min) in a 1% agarose gel containing 0.5 μg·g^{-1} ethidium bromide.

Detection of *abi* genes by PCR

Polymerase chain reaction (PCR) was performed by a conventional procedure using the temperature programme of 5 min at 94 °C followed by thirty cycles (40 s denaturation step at 94 °C, 40 s annealing step at 56 °C and 40 s polymerization step at 72 °C) and a final step of 5 min at 72 °C in Techne Thermal Cycler (Techne, Burlington, New Jersey, USA). Oligonucleotide primer sequences for amplification of all known Abi systems from lactococci (AbiA, AbiB, AbiC, AbiD, AbiD1, AbiEi, AbiEii, AbiF, AbiGi, AbiGii, AbiH, AbiI, AbiJ, AbiK, AbiLi, AbiLii, AbiN, AbiO, AbiP, AbiQ, AbiR, AbiU1, AbiU2) were taken from Miklic and Rogelj [15]. Taq polymerase and other PCR reagents were purchased from Jena Bioscience (Jena, Germany). PCR products were separated by electrophoresis in 1.0% agarose gel using TAE (Tris-acetate-EDTA) buffer and visualised by Gel Logic 212 PRO Imaging System (Carestream, Rochester, New York, USA).

RESULTS AND DISCUSSION

Isolation and identification of bacterial isolates

Multiple bacterial isolates were collected from bryndza cheese on non-selective media and identified using MALDI-TOF mass spectroscopy. MALDI-TOF spectra consisting from 10 to 30 dominant peaks were obtained from the cell lysates of 68 bacterial strains. The majority of peaks were detected in the m/z range from 3000 to 10000. Automated analysis of the raw spectral data was performed with the use of a library of 4613 spectra (database version 3.3.1.0, Bruker Daltonics) and the default settings. Based on the identification score, the isolates from bryndza sheep were identified as *Lactococcus lactis*, *Lactobacillus* spp. and *Enterococcus* spp. For most isolates, secure identification at species level was obtained, with identification scores over 2.3 (data not shown).

Lactococcal population in the ewes' cheese was found to be represented by *Lc. lactis* (24 isolates, 35.3%) only (Tab. 1). In several cheeses produced from raw milk, lactococci were observed as predominant LAB [16, 17]. In the Leben cheese, a traditional cheese made from fermented milk in Algeria [18], and in Pecorino Sardo, a semi-cooked cheese from Sardinia [19], *Lc. lactis* was found to be a single lactococcal species detected, similar to our study.

Lactobacillus population was found to be dominated by *Lb. plantarum* (16 isolates, 23.5%), followed by *Lb. paracasei* (6 isolates, 8.8%). Many species of mesophilic lactobacilli have been isolated from cheese, but those most frequently encountered were *Lb. casei*/*Lb. paracasei, Lb. plantarum, Lb. rhamnosus* and *Lb. curvatus* [20]. Data for many of the cheeses studied indicate a change in the dominant species during ripening, with *Lb. paracasei* becoming dominant later [21]. Recently, Belicova et al. [22] evaluated probiotic potential and safety properties of lactobacilli from bryndza cheese. Out of 26 strains tested, 20 were identified as *Lb. plantarum* and 6 as *Lb. fermentum* [22].

Enterococcal population was found to be dominated by *E. faecium* (16 isolates, 23.5%), followed by *E. durans* (4 isolates, 5.9%) and *E. faecalis* (2 isolates, 3,0%) (Tab. 2). In the survey involving 4379 bacterial isolates from 25 European cheeses, enterococci represented 14% of isolates [23]. In cows' milk and cheese, *E. casseliflavus* was found to be dominant [24], although *E. faecalis* and *E. faecium* were reported to be the most common species of *Enterococcus* in cheese [23]. In the ewes' milk cheese, high amounts of enterococci were reported, ranging between 10^7 CFU

and 10^8 CFU per gram of cheese [25]. Authors reported very similar composition of enterococcal microflora of ewes' cheese when, out of 308 *Enterococcus* sp. strains, 57.5% isolates were *E. faecium*, 19.1% *E. durans*, 13.3% *E. faecalis*, 4.2% *E. mundtii* and 3.6% *E. casseliflavus* [25].

Bacteriophage sensitivity

The double layer agar technique was used to test the sensitivity of isolated bacteria to bacteriophage infections. During incubation, uninfected bacteria multiply to form a confluent film of growth over the surface of the plate. Each infected bacterium, however, bursts after a short time and liberates progeny phages that, in turn, infect adjacent bacteria, which are then lysed. This chain reaction spreads in circular motion until brought to a halt when all bacterial cells have been infected and lysed. The result is a visible, circular area of clearing in the confluent bacterial growth, known as a plaque.

All strains were tested for the sensitivity to the corresponding bacteriophage. *Lb. plantarum* virulent siphophage, ATCC 8014-B1, was originally isolated from maize silage and anaerobic sewage sludge, and it was found to be able to infect 3 of 8 *Lb. plantarum* strains [26]. *Lc. lactis* bacteriophage P008 is accepted as the official type phage for *Lc. lactis* representing the *Siphoviridae* family of morphotype B1 P008. Related species are responsible for important loss each year in cheese factories. The *E. faecalis* MSF1 bacteriophage was isolated from the raw milk and partially characterized in Institute of Animal Physiology Slovak Academy of Science (Košice, Slovakia). The bacteriophage produces small turbid plaques at *E. faecalis* 47/3 host strain. The genome of bacteriophage is formed by double stranded DNA in size between 40 kbp to 50 kbp. MSF1 bacteriophage is the first *E. faecalis* bacteriophage isolated from milk, being closely related to *E. faecalis* bacteriophages and prophages isolated from other environments [12].

Relatively low occurrence of bacteriophage resistance was observed in LAB isolates from bryndza cheese. Eleven out of twenty-six *Lc. lactis* strains were resistant to the *Lb. plantarum* ATCC 8014-B1 bacteriophage (Tab. 1). None of lactobacilli was resistant to the *Lc. lactis* bacteriophage P008 (data not shown), and only four of twenty-two tested enterococci showed resistance to the bacteriophage MSF1 infections (Tab. 2). Surprisingly, *Lb. plantarum* ATCC 8014-B1 and *Lc. lactis* bacteriophage P008 were able to infect some of lactococcal and lactobacilli strains (e.g. *L. lactis* 29S4, data not shown). Although bacteriophages are known to be host-specific, some studies indicated that bacteriophages of *Lb. plantarum* were able to infect other species of bacteria from the same environment [27].

Tab. 1. Presence of abortive infection systems and phage resistance in *Lactococcus lactis* strains from bryndza ewes' cheese.

Species and strain identification		Abi detected	Resistance against bacteriophage	
			LP phage	P008
Lc. lactis	29S4	AbiI	–	–
	37S2	AbiH	–	–
	28S2	none	–	–
	33S3	none	–	–
	33S7	none	–	–
	34S1	none	–	–
	34S2	none	+	–
	34S5	none	–	–
	LM25_A	none	–	–
	LM25_B	none	–	–
	LM25_C	none	+	–
	LM25_D	AbiB	+	+
	LM25_E	AbiB	–	–
	LM25_G	AbiB	+	–
	LM25_H	none	–	–
	LM25_I	none	–	–
	LM25_N	AbiB	–	–
	ZS25_4	AbiB	+	–
	ZS25_5	AbiB	+	–
	ZS25_6	AbiB	+	–
	ZS25_7	AbiB	+	–
	ZS25_9	AbiB	+	–
	ZS25_11	AbiB	+	–
	ZS25_12	AbiB	+	–

Abi – abortive infection system, (+) – denotes resistance against bacteriophage infection, (–) – denotes sensitivity to the bacteriophage infection.

Presence of abortive infection systems

In order to detect the presence of abortive infection systems, genomic DNA from all tested strains was isolated and subsequently used as a template for PCR reactions using 23 sets of specific Abi-oriented primers [15]. Very low frequency of *abi* determinants was detected in the tested isolates (Tab. 1). None of the tested isolates was found to possess AbiA, AbiD1, AbiEii, AbiGi, AbiGii, AbiH, AbiJ, AbiK, AbiLi, AbiLii, AbiN, AbiO, AbiP, AbiQ, AbiR, AbiU1 or AbiU2

Tab. 2. Presence of abortive infection systems and phage resistance in *Enterococcus* spp. strains from bryndza cheese.

Species and strain identification		Abi detected	Resistance against MSF1 bacteriophage
E. faecium	3S1	AbiC, AbiF	–
	3S7	AbiD, AbiEi	–
	5S9	none	–
	6S3	none	–
	7S3	AbiI	+
	7S7	AbiD, AbiF	–
	8S3	AbiF	+
	9S3	AbiF	–
	9S8	none	–
	10S2	AbiEi	+
	10S5	none	–
	12S5	AbiEi	–
	21S1	AbiD	+
	24S10	none	–
	36S3	none	–
	36S5	none	–
E. faecalis	5S10	AbiC	–
	7S9	AbiC, AbiF	–
E. durans	21S10	AbiF	–
	22S1	none	–
	23S2	AbiF	–
	23S6	AbiEi	–

Abi – abortive infection system, (+) – denotes resistance against bacteriophage infection, (–) – denotes sensitivity to the bacteriophage infection.

system, and none of the tested Abi systems was detected in lactobacilli. In *Lc. lactis*, one Abi system was detected in 13 out of 24 strains. The low frequency of Abi systems is unexpected, as almost all lactococcal Abi systems are plasmid-encoded [15]. Dairy lactococci contain many plasmids, representing a significant part of their genetic information. These plasmids carry most of the genes required for optimal growth in milk and they are therefore regarded as major contributors to the adaptation of lactococci to the dairy environment.

The *abiB* genetic determinant was most frequently detected in *Lc. lactis*. Parreira et al. [4] speculated that AbiB may act by having latent RNase activity, which is stimulated by a phage product, by interacting with an early phage product to activate a bacterial RNase, or by activating a phage-encoded RNase. In strain *Lc. lactis* 29S4, we detected *abiI* gene. Su et al. [28] showed that *abiI* gene is encoded by pND852, a 56 kb plasmid. The system consists of a single open reading frame (ORF) and the gene encoding the 322 amino acid AbiI protein. The strain *Lc. lactis* 37S2 possessed a genetic determinant of AbiH system. Prévots et al. [29] showed that this is a chromosomally encoded abortive infection system. According to that study, the ABiH system reduced the formation of plaques of bacteriophage φ53 and provided complete resistance to bacteriophage φ59.

The highest occurrence and variability of abortive infection systems was observed in enterococci (Tab. 2). Genes for five different Abi systems were detected (*abiC*, *abiD*, *abiEi*, *abiF* and *abiI*) and at least one system was detected in 14 out of 22 strains tested (Tab. 2). In 4 tested strains, simultaneous presence of two systems was detected.

The mechanisms of abortive bacteriophage infection was described for many bacterial species, such as *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Vibrio cholerae* or *Lactococcus lactis* [5], which indicates that they are most probably widespread in bacteria. However, their occurrence in the *Enterococcus* genus has not been reported yet.

It is generally accepted that Abi systems are capable of providing bacterial population protection against phage infection. Surprisingly, for most isolates tested, no clear correlation was found between the possession of abortive infection systems and the resistance against bacteriophages. Frequently, strains positive for Abi systems were infected by bacteriophages and, on the other hand, resistance against bacteriophages was observed in isolates without any known Abi systems. However, among 11 *Lc. lactis* strains positive for AbiB system, only two were sensitive to the *Lb. plantarum* ATCC 8014-B1 bacteriophage, while, among 11 *Lc. lactis* strains lacking any Abi system, only two were resistant to this bacteriophage. The possible explanation of this observation is either that some of Abi systems detected were not expressed in a functional form, or, more probably, at least some of Abi systems were able to protect bacteria only against a particular group of bacteriophages. For example, lactococcal phages described to date fall into three well-established groups based on DNA homology and a given Abi system can be active against phages from one, two or three groups [5].

CONCLUSION

A limited number of lactococi and lactobacilli isolated from bryndza cheese was found to possess

genes encoding for Abi systems. Neither of the tested 23 Abi systems was detected in lactobacilli. The most frequently detected system was AbiB in *Lc. lactis*. The highest frequency and variability of abortive infection systems was observed in enterococci isolated from bryndza cheese, where five different Abi systems were detected (AbiC, AbiD, AbiEi, AbiF and AbiI) and at least one system was detected in 14 out of 22 strains tested. In 4 tested strains, simultaneous presence of two systems was detected. When all strains were tested for the sensitivity to the corresponding bacteriophage, no clear correlation was found between the possession of abortive infection systems and the resistance against bacteriophages. Strains positive for Abi systems were infected by bacteriophages and resistance against bacteriophages was observed in isolates without any known Abi system. Multiple phage resistance systems are known in LAB and further study including higher number of bacteriophages will be neccessary to understand the role of bacteriophage defence system in LAB.

Acknowledgements

This publication is the result of the project No. ITMS 26220220065 implementation supported by the Research & Development Operational Programme funded by the ERDF.

REFERENCES

1. Javed, I. – Ahmed, S. – Ali, M. I. – Ahmad, B. – Ghumro, P. B. – Hameed, A – Chaudry, G.: Bacteriocinogenic potential of newly isolated strains of *Enterococcus faecium* and *Enterococcus faecalis* from dairy products of Pakistan. Journal of Microbiology and Biotechnology, *20*, 2010, pp. 153–160. DOI: 10.4014/jmb.0904.04024.
2. Emond, E. – Moineau, S.: Bacteriophages and food fermentations. In: McGrath, S. – van Sinderen, D. (Eds.): Bacteriophage: genetics and molecular biology. Poole : Caister Academic Press 2007, pp. 93–124. ISBN: 978-1-904455-14-1.
3. Garneau, J. E. – Moineau, S.: Bacteriophages of lactic acid bacteria and their impact on milk fermentations. Microbial Cell Factories, *10*, 2011, pp. 1–20. DOI: 10.1186/1475-2859-10-S1-S20.
4. Parreira, R. – Ehrlich, S. D. – Chopin, M. C.: Dramatic decay of phage transcripts in lactococcal cells carrying the abortive infection determinant AbiB. Molecular Microbiology, *19*, 1996, pp. 221–230. DOI: 10.1046/j.1365-2958.1996.371896.x.
5. Chopin, M. C. – Chopin, A. – Bidnenko, E.: Phage abortive infection in lactococci: variations on a theme. Current Opinion in Microbiology, *8*, 2005, pp. 473–479. DOI: 10.1016/j.mib.2005.06.006.
6. Prevots, F. – Ritzenthaler, P.: Complete sequence of the new lactococcal abortive phage resistance gene abiO. Journal of Dairy Science, *81*, 1998, pp. 1483–1485. DOI: 10.3168/jds.S0022-0302(98)75713-3.
7. McLandsborough, L. A. – Kolaetis, K. M. – Requena, T. – McKay, L. L.: Cloning and characterization of the abortive infection genetic determinant *abiD* isolated from pBF61 of *Lactococcus lactis* subsp. *lactis* KR5. Applied and Environmental Microbiology, *61*, 1995, pp. 2023–2026.
8. O'Connor, L. – Coffey, A. – Daly, C. – Fitzgerald, G. F.: AbiG, a genotypically novel abortive infection mechanism encoded by plasmid pCI750 of *Lactococcus lactis* subsp. *cremoris* UC653. Applied and Environmental Microbiology, *62*, 1996, pp. 3075–3082.
9. Djordjevic, G. M. – O'Sullivan, D. J. – Walker, S. A. – Conkling, M. A. – Klaenhammer, T. R.: A triggered-suicide system designed as a defense against bacteriophages. Journal of Bacteriology, *179*, 1997, pp. 6741–6748.
10. Coffey, A. – Ross, R. P.: Bacteriophage-resistance systems in dairy starter strains: molecular analysis to application. Antonie van Leeuwenhoek, *82*, 2002, pp. 303–321. DOI: 10.1023/A:1020639717181.
11. Biswas, S. – Rolain, J. M.: Use of MALDI-TOF mass spectrometry for identification of bacteria that are difficult to culture. Journal of Microbiological Methods, *92*, 2013, pp. 14–24. DOI: 10.1016/j.mimet.2012.10.014.
12. Šulak, M. – Tomáška, M. – Kološta, M. – Javorský, P. – Pristaš, P.: The partial characterization of *Enterococcus faecalis* MSF1 bacteriophage from raw milk. Journal of Microbiology, Biotechnology and Food Sciences, *3*, 2014, pp. 505–508.
13. Maniatis, T. – Fritsch, E. F. – Sambrook, J.: Molecular cloning: A laboratory manual. New York : Cold Spring Harbor Laboratory, 1982. ISBN: 0-87969-136-0.
14. Adams, M. H.: Bacteriophages. New York : Interscience Publishers, 1959. OCLC 326505.
15. Miklic, A. – Rogelj, I.: Screening for natural defence mechanisms of *Lactococcus lactis* strains isolated from traditional starter cultures. International Journal of Food Science and Technology, *42*, 2000, pp. 777–782. DOI: 10.1111/j.1365-2621.2007.01175.x.
16. Bonetta, S. – Carraro, E. – Rantsiou, K. – Cocolin, L.: Microbiological characterization of Robiola di Roccaverano cheese using PCR-DGGE. Food Microbiology, *25*, 2008, pp. 786–792. DOI: 10.1016/j.fm.2008.04.013.
17. Flórez, A. B. – de los Reyes-Gavilán, C. G. – Wind, A. – Mayo, B. – Margolles, A. A.: Ubiquity and diversity of multidrug resistance genes in *Lactococcus lactis* strains isolated between 1936 and 1995. FEMS Microbiology Letters, *263*, 2006, pp. 21–25. DOI: 10.1111/j.1574-6968.2006.00371.x.
18. Bensalah, F. – Delorme, C. – Renault, P.: Characterisation of thermotolerant cocci from indigenous flora of "Leben" in Algerian arid area and DNA identification of atypical *Lactococcus lactis* strains. Current Microbiology, *59*, 2009, pp. 139–146. DOI: 10.1007/s00284-009-9411-1.

19. Mannu, L. – Paba, A.: Genetic diversity of lactococci and enterococci isolated from home-made Pecorino Sardo ewes' milk cheese. Journal of Applied Microbiology, *92*, 2002, pp. 55–62. DOI: 10.1046/j.1365-2672.2002.01489.x.
20. Beresford, P. T. – Fitzimons, N. A. – Brennan, N. L. – Cogan, T. M.: Recent advances in cheese microbiology. International Dairy Journal, *11*, 2001, pp. 259–274. DOI: 10.1016/S0958-6946(01)00056-5.
21. Fitzsimons, N. A. – Cogan, T. M. – Condon, S. – Beresford, T.: Spatial and temporal distribution of non-starter lactic acid bacteria in Cheddar cheese. Journal of Applied Microbiology, *90*, 2001, pp. 600–608. DOI: 10.1046/j.1365-2672.2001.01285.x.
22. Belicová, A. – Mikulášová, M. – Dušinský, R.: Probiotic potential and safety properties of *Lactobacillus plantarum* from Slovak bryndza cheese. BioMed Research International, 2013, Article ID 760298. DOI:10.1155/2013/760298.
23. Cogan, T. M. – Barbosa, M. – Beuvier, E. – Bianchi-Salvadori, B. – Cocconcelli, P. S. – Fernandes, I. – Gomez, J. – Gomez, R. – Kalantzopoulos, G. – Ledda, A. – Medina, M. – Rea, M. C. – Rodriguez, E.: Characterisation of the lactic acid bacteria in artisanal dairy products. Journal of Dairy Research, *64*, 1997, pp. 409–421. DOI: 10.1017/S0022029997002185.
24. Gelsomino, R. – Vancanneyt, M. – Condon, S. – Swings, J. – Cogan, T. M.: Enterococcal diversity in the environment of an Irish Cheddar-type cheesemaking factory. International Journal of Food Microbiology, *71*, 2001, pp. 177–188. DOI: 10.1016/S0168-1605(01)00620-1.
25. Jurkovič, D. – Križková, L. – Dušinský, R. – Belicová, A. – Sojka, M. – Krajčovič, J. – Ebringer, L.: Identification and characterization of enterococci from bryndza cheese. Letters in Applied Microbiology, *42*, 2006, pp. 553–559. DOI: 10.1111/j.1472-765X.2006.01918.x.
26. Marcó, M. B. – Garneau, J. E. – Tremblay, D. – Quiberoni, A. – Moineau, S.: Characterization of two virulent phages of *Lactobacillus plantarum*. Applied and Environmental Microbiology, *78*, 2012, pp. 8719–8734. DOI: 10.1128/AEM.02565-12.
27. Lu, Z. – Breidt, F. – Plengvidhya, V. – Fleming, H. P.: Bacteriophage ecology in commercial sauerkraut fermentations. Applied and Environmental Microbiology, *69*, 2003, pp. 3192–3203. DOI: 10.1128/AEM.69.6.3192-3202.2003.
28. Su, P. – Harvey, M. – Im, H. J. – Dunn, N. W.: Isolation, cloning and characterisation of the abiI gene from *Lactococcus lactis* subsp. *lactis* M138 encoding abortive phage infection. Journal of Biotechnology, *54*, 1997, pp. 95–104. DOI: 10.1016/S0168-1656(97)01692-1.
29. Prévots, F. – Daloyau, M. – Bonin, O. – Dumont, X. – Tolou, S.: Cloning and sequencing of the novel abortive infection gene abiH of *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* S94. FEMS Microbiology Letters, *142*, 1996, pp. 295–299. DOI: 10.1111/j.1574-6968.1996.tb08446.x.

Received 10 July 2014; accepted 4 August 2014; published online 23 January 2015.