OPEN ACCESS
Potravinarstvo® Scientific Journal for Food Industry
Crossref Similarity Check Powered by iThenticate

potravinárstvo®

Potravinarstvo, vol. 10, 2016, no. 1, p. 518-523
doi:10.5219/652
Received: 12 September 2016. Accepted: 13 October 2016.
Available online: 16 October 2016 at www.potravinarstvo.com

ISSN 1337-0960 (online)
License: CC BY 3.0

# SELECTED TECHNOLOGICAL PROPERTIES AND ANTIBIOTIC RESISTANCE OF ENTEROCOCCI ISOLATED FROM MILK

*Čanigová Margita, Ducková Viera, Lavová Monika, Bezeková Jana, Kročko Miroslav*

## ABSTRACT

The aim of this work was to determine counts of enterococci in raw cow milk, to isolate and identify them, to determinate their antibiotic resistance, ability of lactose fermentation, proteolytic and lipolytic activity in different conditions of cultivation. Counts of enterococci were determined after 48 ±2 h cultivation on Slanetz-Bartley agar at 37 ±1 °C. The counts of enterococci in raw cow milk fluctuated from 1.80 x $10^2$ to 1.77 x $10^3$ CFU.mL$^{-1}$ with average value 7.25 x $10^2$ CFU.mL$^{-1}$. Species identifications of enterococci isolates were performed using commercial EN-COCCUS test and confirmed by PCR. Majority of tested isolates (85.7%) was included to species *E. faecalis*. Antibiotic resistance was tested on Mueller-Hinton agar using following antimicrobial discs: vancomycin (VA) 30 µg.disc$^{-1}$, gentamicin (CN) 120 µg.disc$^{-1}$, erythromycin (E) 15 µg.disc$^{-1}$, tetracycline (TE) 30 µg.disc$^{-1}$, ampicillin (AMP) 10 µg.disc$^{-1}$, teicoplanin (TEC) 30 µg.disc$^{-1}$. From 13 isolates of enterococci, 1 strain was resistant to vancomycin, 1 strain to tetracycline and 1 to ampicillin, but higher prevalence of intermediate resistance of isolates was determined to tetracycline (5 strains). Ability of lactose fermentation was monitored by change of titratable acidity in UHT milk after 0, 18, 24, 40 and 48 h of cultivation at temperature 25, 30 and 37 °C. The tested strains of enterococci exhibit low milk acidifying ability. Production of proteolytic enzymes was evaluated after cultivation at temperature 7, 25 and 30 °C after 10 days on nutrient agar no. 2 with sterile skim milk (10% w/v) with pH 6.0 and 6.5. Proteolytic activity of tested enterococci strains varied depending on tested temperature and pH. Lipolytic activity was determined similarly like proteolytic activity but on tributyrin agar base with tributyrin (1% w/v). Lipolytic activity of isolated enterococci was very low. The tested strains produced halos with zone in range from 7 to 15 mm regardless of pH, cultivation time and temperature. Some of isolated and tested enterococci strains have shown suitable technological properties, but they have exhibited resistance to antibiotic.

**Keywords:** enterococci; milk; lactose fermentation; proteolytic and lipolytic activity; antibiotic resistance

## INTRODUCTION

Enterococci are Gram-positive, non-sporeforming, catalase-negative, oxidase-negative, facultative anaerobic cocci that occur singly, in pairs, or in chains **(Hollenbeck and Rice, 2012)**. Most enterococcal species are able to grow in the presence of 6.5% NaCl, 40% bile salts, at pH 9.6 **(Ogier and Serror, 2008)**, at 10 and 45 °C and survive for at least 30 min at 60 °C **(Domig et al., 2003)**. Enterococci are ubiquitous bacteria which occur in many different habitats such as in soil, surface water, ocean water, sewage, on plants and in the gastrointestinal tract of animals and humans. Based on their association with the gastrointestinal tract, enterococci often occur in foods of animal origin such as meat, fermented and cooked meat, as well as cheese **(Franz et al., 2011)**.

Enterococci are normal components of the raw milk microbiota **(Giménez-Pereira, 2005)** and pasteurised milk microflora. Due to their psychrotrophic nature, their heat resistance and their adaptability to different substrates and growth conditions, count of enterococci can increase during milk refrigeration and survive after pasteurisation **(Giraffa, 2003)**.

Presence of enterococci in dairy products can have conflicting effects, of either a risk as a foreign or intrusive flora indicating poor hygiene during milk handling and processing (if in excessive numbers), or as a benefit in contributing to produce unique traditional and emerging by-products, in protecting against diverse spoilers, and as probiotics **(Giménez-Pereira, 2005)**.

Enterococci possess intrinsic antibiotic resistance to cephalosporins, ß-lactams, sulphonamides, and to certain levels of clindamycin and aminoglycosides, while acquired resistance exists to chloramphenicol, erythromycin, clindamycin, aminoglycosides, tetracycline, ß-lactams, fluoroquinolones **(Giménez-Pereira, 2005)** and glycopeptide antibiotics (vancomycin and teicoplanin) **(Cariolato et al., 2008)**.

On the other hand certain enterococcal strains are also successfully used as probiotics to improve human or animal health **(Araújo and Ferreira, 2013)**. *Enterococcus* main characteristic is the ability to produce L-lactic acid (lactate) from hexoses by means of homofermentative lactic acid fermentation. Although the main product is lactate, they can also produce significant amounts of acetate, formate **(Rea and Cogan, 2003)**. Acetate and the others are recognised as "flavour compounds" since they are important in determining the taste of many fermented dairy products **(Battelli et al., 2011)**. Enterococci

contribute to texture and aroma development of cheeses also thanks to their proteolytic and lipolytic activities **(Martín-Platero et al., 2009)**.

The aim of this work was to determine counts of enterococci in raw cow milk from milk machines, to isolate and identify them, to determinate their antibiotic resistance, ability of lactose fermentation, proteolytic and lipolytic activity in different conditions of cultivation.

## MATERIAL AND METHODOLOGY

Ten samples of raw cow milk were obtained from the milk machines. The counts of enterococci were determined by cultivation on Slanetz-Bartley agar (HiMedia Laboratories, India) at 37 ±1 °C after 48 ±2 h. Suspect colonies of enterococci (n = 38) were incubated on bile esculin azide agar (BEAA) (Biokar Diagnostic, France) at 37 ±1 °C for 24 ±2 h **(STN 56 0100, 1970)** for evaluation their hydrolysis. Then the isolates were identified using optical microscopy, catalase production and by PYR test (Lachema, Czech Republic). Species identification was performed using commercial EN-COCCUS test (Lachema, Czech Republic) and confirmed by PCR **(Kariyama et al., 2000)**.

Antibiotic resistance was tested on Mueller-Hinton agar (HiMedia Laboratories, India) using following antimicrobial discs: vancomycin (VA) 30 μg.disc$^{-1}$, gentamicin (CN) 120 μg.disc$^{-1}$, erytromycin (E) 15 μg.disc$^{-1}$, tetracycline (TE) 30 μg.disc$^{-1}$, ampicillin (AMP) 10 μg.disc$^{-1}$, teicoplanin (TEC) 30 μg.disc$^{-1}$ (HiMedia Laboratories, India). The isolates were classified as susceptible, intermediate resistant and resistant according **CLSI criteries (2013)**.

Then lactose fermentation, production of proteolytic and lipolytic enzymes was determined. Enterococci strains were incubated on glucose tryptone yeast extract agar (HiMedia Laboratories, India) at 37 °C for 24 ±2 h. The inoculum was prepared by suspending of enterococcal colonies in saline and by adjusting to equal 0.5 McFarland standard using Densilameter (Lachema, Czech Republic).

Ability of lactose fermentation was monitored by change of titratable acidity in UHT milk (100 mL) with 1 mL of inoculum. It was cultivated at temperature 25, 30 and 37 °C. The titratable acidity was reported immediately after inoculation and after 18, 24, 40 and 48 h of cultivation.

The inoculated UHT milk after 48 h of cultivation at 7, 25 and 30 °C was used for detection of proteolytic activity of enterococci. It was determined using hole diffusion method on nutrient agar no. 2 (HiMedia Laboratories, India) with sterile skim milk (10% w/v), with pH value 6.0 and 6.5. The inoculated UHT milk (30 μL) was applied into the hole in medium. Production of proteolytic enzymes was evaluated after cultivation at temperature 7, 25 and 30 °C after 10 days.

Lipolytic activity was determined similarly like proteolytic activity but on tributyrin agar base (HiMedia Laboratories, India) with tributyrin (1% w/v).

## RESULTS AND DISCUSSION

Enterococci are commonly found in raw milk with different flora reported in different countries, reflecting local practices and levels of hygiene. The counts of enterococci in our samples of raw cow milk ranged from 1.80 x $10^2$ to 1.77 x $10^3$ CFU.mL$^{-1}$ with average value 7.25 x $10^2$ CFU.mL$^{-1}$.

**Giménez-Pereira (2005)** determined higher values in raw cow milk, counts of enterococci fluctuated from $10^3$ to $10^5$ CFU.mL$^{-1}$. In the study of **Fabianová et al. (2010)** were presented counts of enterococci in cistern samples from 1.3 x $10^3$ to 2.9 x $10^4$ CFU.mL$^{-1}$ and in the samples from storage tank from 2.1 x $10^3$ to 3.2 x $10^4$ CFU. mL$^{-1}$. **McAuley et al. (2015)** detected enterococci in 96% of the raw milk samples (detection limit 1 log CFU.mL$^{-1}$), with counts ranging from <1 to 6.80 log CFU.mL$^{-1}$ with an average of 2.48 log CFU.mL$^{-1}$; most counts (77.3%) were <3 log CFU.mL$^{-1}$.

Sources tracking entry of enterococci into raw milk and subsequent transmission into processed products have indicated persistence of particular species of strain types, where the likely source of contamination of these is through milking process and processing equipment, as well

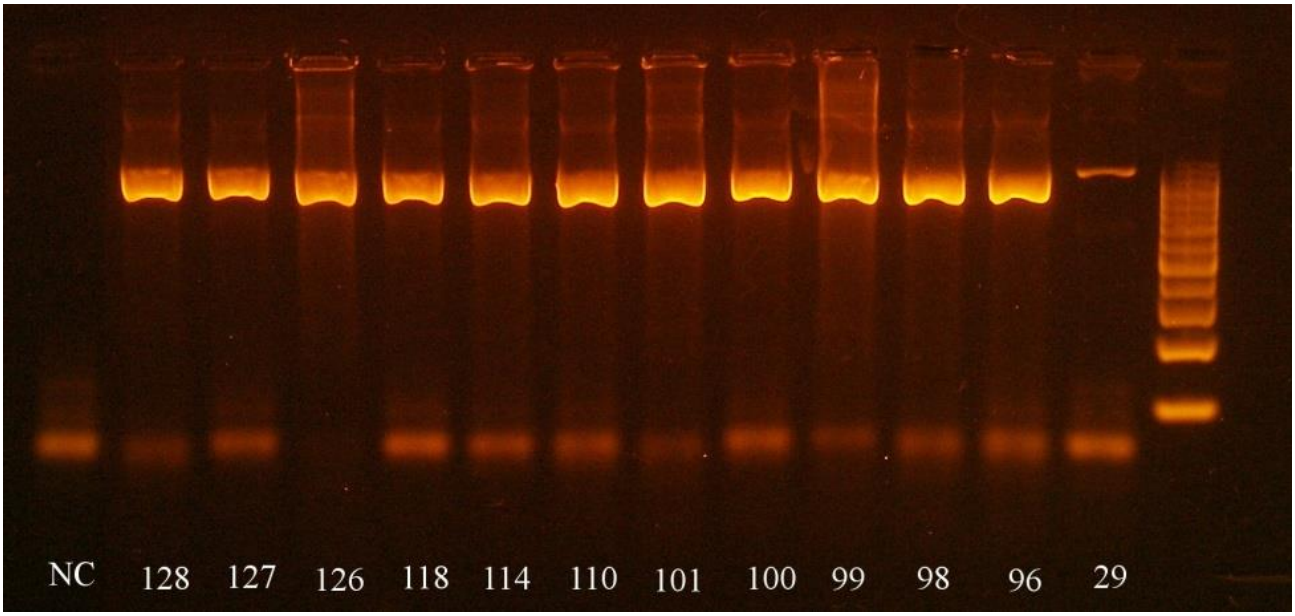


**Figure 1** PCR identification of enterococci strains.

**Table 1** Evaluation of antibiotic resistance of enterococci isolated from raw cow milk according CLSI (2013).

| Number of strain | antibiotics | | | | | |
|---|---|---|---|---|---|---|
| | VA | CN | E | TE | AMP | TEC |
| **29** | S | S | S | S | S | S |
| **96** | R | S | S | I | S | S |
| **98** | S | S | S | R | S | S |
| **99** | S | S | S | S | S | S |
| **100** | S | S | S | I | R | S |
| **101** | S | S | S | I | S | S |
| **108** | S | S | R | I | S | S |
| **110** | S | S | S | S | S | S |
| **114** | S | S | S | I | S | S |
| **118** | S | S | S | S | S | S |
| **126** | S | S | S | S | S | S |
| **127** | S | S | S | S | S | S |
| **128** | S | S | S | S | S | S |

NOTE: VA – Vancomycin (30 µg.disc$^{-1}$), CN – Gentamicin (120 µg. disc$^{-1}$), E – Erythromycin (15 µg. disc$^{-1}$), TE – Tetracycline (30 µg. disc$^{-1}$), AMP – Ampicillin (10 µg. disc$^{-1}$), TEC – Teicoplanin (30 µg. disc$^{-1}$), S – susceptible, I – intermediate resistant, R – resistant.

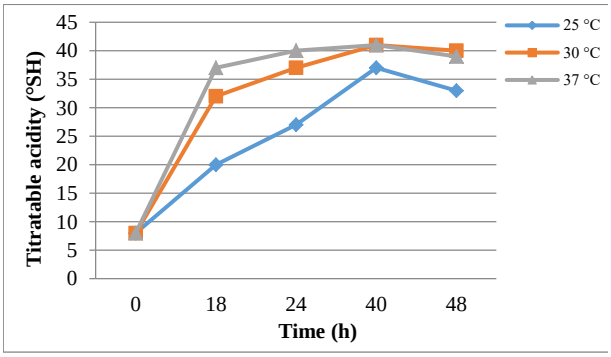


**Figure 2** Acidifying activity of strain No. 98 in dependence on time and temperature.

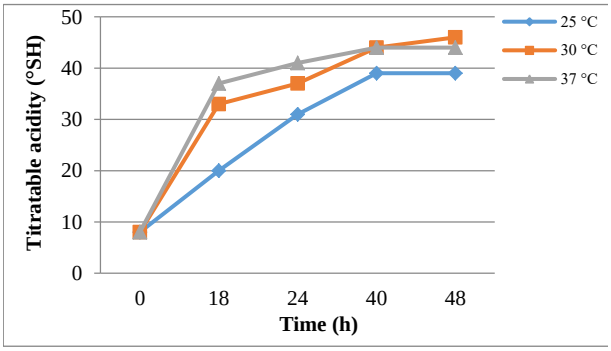


**Figure 3** Acidifying activity of strain No. 100 in dependence on time and temperature.

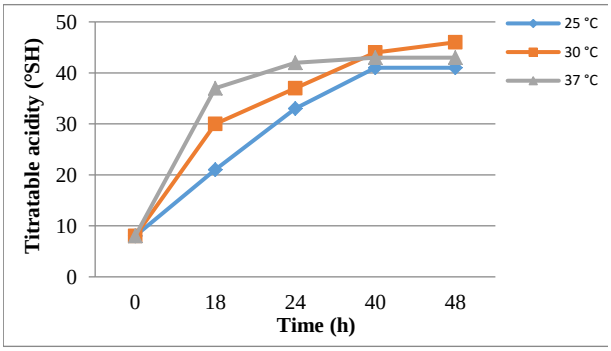


**Figure 4** Acidifying activity of strain No. 118 in dependence on time and temperature.

The species identification of enterococci was determined by EN-COCCUS test. Majority of tested isolates (85.7%) was included to species *E. faecalis*. One strain (no 29) was classified as *E. durans* and one strain was not included to *Enterococcus* spp. Results of EN-COCCUS test were confirmed using PCR method (Figure 1).

**McAuley et al. (2012)** observed also a low prevalence of the more thermoduric species in the raw milk (*E. faecium*, *E. hirae*, and *E. durans*).

Several studies suggest that *E. faecalis* is the dominant species of the genus *Enterococcus* in raw milk. For example, **Fabianová et al. (2010)** determined species *E. faecalis*, *E. faecium*, *E. group III.*, *E. mundtii* and *E. casseliflavus* by EN-COCCUS test in samples of raw cow milk. Species *E. faecalis* represented dominant part of all isolates (56.5%). Also according **McAuley et al. (2015)** *E. faecalis* was the most prevalent species isolated from raw milk, comprising between 61.5 and 83.5% of enterococcal species across each season.

From 13 isolates of enterococci, 1 strain was resistant to vancomycin, 1 strain to tetracycline and 1 to ampicillin, but higher prevalence of intermediate resistance of isolates was determined to tetracycline (5 strains) (Table 1). In the study of **Ruiz et al. (2016)** *E. faecium*, *E. faecalis* and *E. hirae* showed a high percentage of resistance to tetracycline. However, in a research of **Gaglio et al. (2016)**, the strains exhibited high percentages of resistance to erythromycin (52.5%), ciprofloxacin (35.0%), quinupristin-dalfopristin (20.0%), tetracycline (17.5%) and high-level streptomycin (5.0%).

In a study of **Nam et al. (2010)** was detected that 105 of enterococci isolates were more resistant to tetracycline (69.5%) than penicillin (64.7%), erythromycin (57.1%) and cephalotine (44.7%). According to **Trivedi et al. (2011)**, from 250 isolates of enterococci 46% were resistant to cephalotine and 38% to ofloxacine. Low resistance was determined to ampicillin, chloramphenicol, gentamicin and teicoplanin. In this study was shown that strains *E. faecalis* and *E. faecium* were resistant the most frequently. Also in the results of **Valenzuela et al. (2009)**, the strains *E. faecalis* isolated from milk and cheeses were the most frequent species of genus *Enterococcus* resistant to antibiotics.

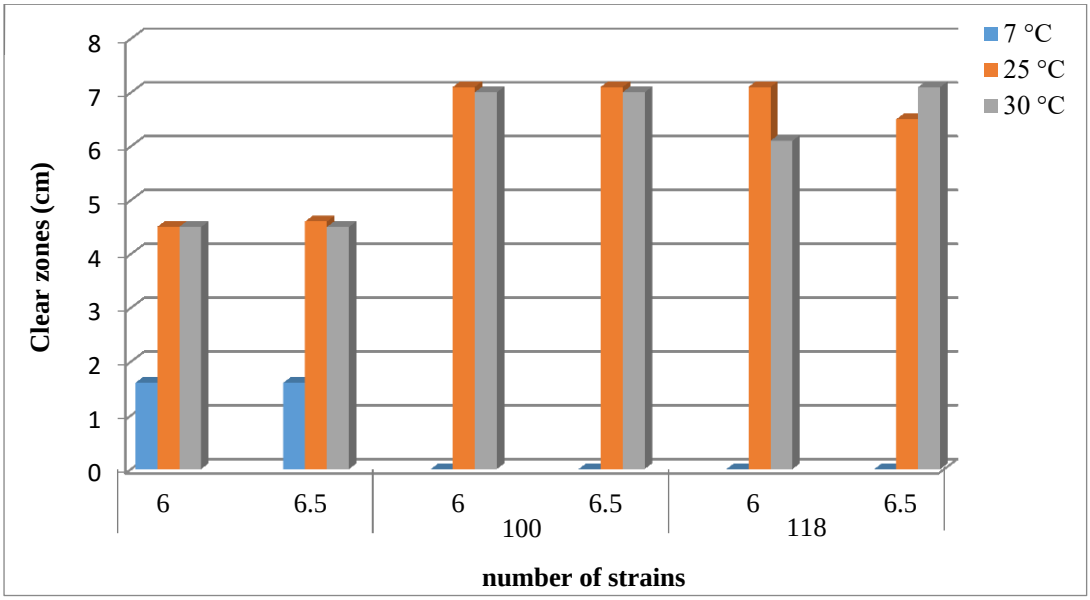


**Figure 5** Proteolytic activity of enterococci after 10 days of cultivation at different pH (6.0 and 6.5) and temperature.

Ability of lactose fermentation was evaluated in all identified strains. The most active strains of enterococci (no. 98, 100 and 118 – *E. faecalis*) are shown in Figure 2, Figure 3 and Figure 4, where are presented changes of titratable acidity of inoculated UHT milk in dependence on time and temperature.

The highest increase of titratable acidity was observed after 18 hour of cultivation at temperature 37 °C and the highest values of titratable acidity were observed after 40 hour of cultivation at both temperatures 30 and 37 °C. The lowest values of titratable acidity were reached after cultivation at 25 °C.

In general, enterococci exhibit low milk acidifying ability. According to **Morea et al. (1999)** the pH of milk 24 hour after inoculation with strains of enterococci isolated from Mozzarella

The poor acidifying capacity of enterococci isolated from food of dairy origin was confirmed also by **Durlu-Ozkaya et al. (2001)**, **Morandi et al. (2006)**, **Serio et al. (2010)** and **Aspri et al. (2016)**.

Acidifying activity was weak, while interesting differences were found for proteolytic capability. The proteolytic system of LAB (including genera *Enterococcus*) is essential for the optimal growth in milk through the release of proteolytic enzymes. LAB have a complex system of proteases and peptidases, which allow them to use milk casein as a source of amino acids and nitrogen. Intra- and inter-specific variability in proteolysis is commonly reported for isolates from natural sources **(Franciosi et al., 2009)**.

Proteolytic activity of tested enterococci strains varied depending on tested temperature and pH value. In Figure 5 are shown proteolytic activities of strains no. 98, 100 and 118. The lowest production of proteolytic enzymes was determined in strain no. 98. No proteolytic activity was determined in strains no. 100 and 118 at 7 °C, in contrast with strain no. 98. The highest values of proteolytic activity were determined in strain 100.

**Gardini et al. (2001)** found out the maximum proteolysis of *E. faecalis* at an incubation temperature 32 – 34 °C. The effect of pH value on this activity was rather weak (at least within the interval of values considered in this investigation). In a study of **Serio et al. (2010)** proteolytic activity was higher at 10 °C than at 30 °C, possibly due to the prolonged incubation time. Especially after 15 days, *E. faecalis* was the most active species. According to **Aspri et al. (2016)** 78% of tested enterococci have shown positive result for proteolytic activity after 4 days of cultivation at 37 °C.

Lipolysis is an important process mainly in cheese ripening due to its role in the development of flavor and texture of the final product. Lipolytic activity of isolated enterococci was very low. Our strains produced halos with zone in range from 7 to 15 mm regardless of pH value, cultivation time and temperature. Limited reports exist on the lipolytic activity of enterococci with *E. faecalis* being the most lipolytic species, followed by *E. faecium* and *E. durans* **(Giraffa, 2003)**. The results obtained in study of **Aspri et al. (2016)** confirmed that enterococci have generally low lipolytic activity, because none of the enterococci tested gave a zone.

## CONCLUSION

The results of this study demonstrated that raw cow milk is good source of autochthones enterococci. The tested indigenous strains of *Enterococcus* spp. showed interesting technological properties that could potentially be utilized further by the food industry especially in dairy technology (i.e. fermented dairy products and cheeses). Regarding the values of titratable acidity, the isolated strains are capable of producing a mild acid flavor to the fermented milk product and could be used as adjunct cultures. The proteolytic activity of enterococci as a selection criterion for the production of fermented milks may not be as crucial as it is for say, cheese production, but proteolytic strains could lead to the formation of peptides with bioactive properties during milk fermentation. The low lipolytic activity of tested enterococci can be considered as an advantage, because it may cause only a slight lysis of the milk fat without flavor change of final product. Furthermore, in order to assessment suitability of tested

enterococci as adjunct cultures, their safety profile (e.g. susceptibility to antibiotics, the absence of virulence factors and production of biogenic amines) must be also investigated.

## REFERENCES


Araújo, T. F., Ferreira, C. L. L. F. 2013. The genus Enterococcus as probiotic: safety concerns. *Brazilian Archives of Biology and Technology*, vol. 56, no. 3, p. 457-466. http://dx.doi.org/10.1590/S1516-89132013000300014

Aspri, M., Bozoudi, D., Tsaltas, D., Hill, C., Papademas, P. 2016. Raw donkey milk as a source of Enterococcus diversity: Assessment of their technological properties and safety characteristics. *Food Control*, article in press. http://dx.doi.org/10.1016/j.foodcont.2016.05.022

Battelli, T., Silvetti, T., Decimo, M., Brasca, M. 2011. Volatile organic compounds production in milk by *Enterococcus faecalis*. 10th international meeting on mountain cheese 14-15 September 2011, Dronero (CN) Italy, p. 75-76.

Cariolato, D., Andrighetto, C., Lombardi, A. 2008. Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. *Food Control*, vol. 19, no. 9, p. 886-892. http://dx.doi.org/10.1016/j.foodcont.2007.08.019

CLSI: M 100 – S23. 2013. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-third Informational Supplement, M02-A11. Clinical and Laboratory Standards Institute, USA, 206 p.

Domig, K. J., Mayer, H. K., Kneifel, W. 2003. Methods used for the isolation, enumeration, characterization and identification of *Enterococcus* spp.: 1. Media for isolation and enumeration. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p.147-164. http://dx.doi.org/10.1016/s0168-1605(03)00178-8

Durlu-Ozkaya, F., Xanthopoulos, V., Tunail, N., Litopoulou-Tzanetak, E. 2001. Technologically important properties of lactic acid bacteria isolates from Beyaz cheese made from raw ewes' milk. *Journal of Applied Microbiology*, vol. 91, p. 861-870. http://dx.doi.org/10.1046/j.1365-2672.2001.01448.x

Fabianová, J., Ducková, V., Čanigová, M., Kročko, M. 2010. Presence of Enterococci in cow milk and their antibiotic resistance. *Potravinarstvo*, vol. 4, no. 2, p. 17-21. http://dx.doi.org/10.5219/45

Franciosi, E., Settanni, L., Cavazza, A., Poznanski, E. 2009. Biodiversity and technological potential of wild lactic acid bacteria from raw cows' milk. *International Dairy Journal*, vol. 19, p. 3-11. http://dx.doi.org/10.1016/j.idairyj.2008.07.008

Franz, C. M. A. P., Huch, M., Abriouel, H., Holzapfel, W., Gálvez, A. 2011. Enterococci as probiotics and their implications in food safety. *International Journal of Food Microbiology*, vol. 151, no. 2, p. 125-140. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.08.014

Gaglio, R., Couto, N., Marques, C´., Fatima, M., Lopes, S., Moschetti, G., Pomba, C., Settanni, L. 2016. Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials and traditional cheeses. *International Journal of Food Microbiology*, vol. 236, p. 107-114. http://dx.doi.org/10.1016/j.ijfoodmicro.2016.07.020

Gardini, F., Martuscelli, M., Caruso, M. C., Galgano, F., Crudele, M. A., Favati, F., Guerzoni, M. E., Suzzi, G. 2001. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. *International Journal of Food Microbiology*, vol. 64, no. 1-2, p. 105-117. http://dx.doi.org/10.1016/s0168-1605(00)00445-1

Giménez-Pereira, M. L. 2005. Enterococci in milk products: Dissertation Work. New Zealand: Massey University Palmerston North, 123 p.

Giraffa, G. 2003. Functionality of enterococci in dairy products. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p. 215-222. http://dx.doi.org/10.1016/s0168-1605(03)00183-1

Hollenbeck, B. L., Rice, L. B. 2012. Intrinsic and acquiried resistance mechanisms in Enterococcus. *Virulence*, vol. 3, no. 5, p. 421-569. http://dx.doi.org/10.4161/viru.21282

Kagkli, D. M., Vancanneyt, M., Hill, C., Vandamme, P., Cogan, T. M. 2007. Enterococcus and Lactobacillus contamination of raw milk in a farm dairy environment. *International Journal of Food Microbiology*, vol. 114, no. 2, p. 243-251. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.09.016

Karyiama, R., Mitsuhata, R., Chow, J. W., Clewell, D. B., Kumon, H. 2000. Simple a reliable multiple PCR assay for surveillance isolates of vancomycin-resistant Enterococci. *Journal of Clinical Microbiology*, vol. 38, no. 8, p. 3092-3095.

Martín-Platero, A. M., Valdivia, E., Maqueda, M., Martínez-Bueno, M. 2009. Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses. *International Journal of Food Microbiology*, vol. 132, no. 1, p. 24-32. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.03.010

McAuley, M. C., Britz, M. L., Gobius, K. S., Craven, H. M. 2015. Prevalence, seasonality, and growth of enterococci in raw and pasteurized milk in Victoria. *Australia. Journal of Dairy Science*, vol. 98, p. 8348-8358. http://dx.doi.org/10.3168/jds.2015-9335

McAuley, M. C., Gobius, K. S., Britz, M. L., Craven, H. M. 2012. Heat resistance of thermoduric enterococci isolated from milk. *International Journal of Food Microbiology*, vol. 154, no. 3, p. 162-168 http://dx.doi.org/10.1016/j.ijfoodmicro.2011.12.033

Morandi, S., Brasca, M., Andrighetto, Ch., Lombardi, A., Lodi, R. 2006. Technological and molecular characterization of enterococci isolated from north–west Italian dairy products. *International Dairy Journal*, vol. 16, p. 867-875. http://dx.doi.org/10.1016/j.idairyj.2005.09.005

Morea, M., Baruzzi, F., Cocconcelli, P. S. 1999. Molecular and physiological characterization of dominant bacterial populations in traditional Mozzarella cheese processing. *Journal of Applied Microbiology*, vol. 87, p. 574-582. http://dx.doi.org/10.1046/j.1365-2672.1999.00855.x

Nam, H. M., Lim, S. K., Moon, J. S., Kang, H. M., Kim, J. M., Jang, K. C., Kim, J. M., Kang, M. I., Joo, Y. S., Jung, S. C. 2010. Antimicrobial resistance of enterococci isolated from mastic bovine milk samples in Korea. *Zoonoses and public health*, vol. 57, no. 7-8, p. 59-64. http://dx.doi.org/10.1111/j.1863-2378.2009.01307.x

Ogier, J. C., Serror, P. 2008. Safety assessment of dairy microorganisms: The Enterococcus genus. *International Journal of Food Microbiology*, vol. 126, no. 3, p. 291-301. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.08.017

Rea, M. C., Cogan, T. M. 2003. Glucose prevents citrate metabolism by enterococci. *International Journal of Food Microbiology*, vol. 88, no. 2-3, p. 201-206. http://dx.doi.org/10.1016/s0168-1605(03)00181-8

Ruiz, P., Perez-Martin, F., Sesena, S., Palop, M. L. 2016. Seasonal diversity and safety evaluation of enterococci

population from goat milk in a farm. *Dairy Science & Technology*, vol. 96, no. 3, p. 359-375. http://dx.doi.org/10.1007/s13594-015-0273-y

Serio, C., Chaves-López, A., Paparella, A., Suzzi, G. 2010. Evaluation of metabolic activities of enterococci isolated from Pecorino Abruzzese cheese. *International Dairy Journal*, vol. 20, no. 7, p. 459-464. http://dx.doi.org/10.1016/j.idairyj.2010.02.005

*STN 56 0100, 1970. Mikrobiologické skúšanie požívatín, predmetov bežnej spotreby a prostredia potravinárskych prevádzok - Microbiological examination of foodstuffs, articles of current use and environment of food establishments. Bratislava: ÚNMS, 239 p.*

Trivedi, K., Cupáková, S., Karpišková, R. 2011. Virulence factors and antibiotic resistance in enterococci isolated from food-stufs. *Veterinarni Medicina*, vol. 56, no. 7, p. 352-357. http://dx.doi.org/10.17221/vetmed

Valenzuela, A. S., Omar, N. B., Abriouel, H., López, R, L., Ortega, E., Cañamero, M. M., Gálvez, A. 2008. Risk factors in enterococci isolated from foods in Morocco: Determination of antimicrobial resistance and incidence of virulence traits. *Food and Chemical Toxicology*, vol. 46, no. 8, p. 2648-2652. http://dx.doi.org/10.1016/j.fct.2008.04.021

**Acknowledgments:**

This work was supported by grant APVV SK-SRB-2013-0038.

**Contact address:**

Margita Čanigová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department for Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: margita.canigova@uniag.sk.

Viera Ducková, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department for Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: viera.duckova@uniag.sk.

Monika Lavová, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department for Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: monika.lavova@uniag.sk.

Jana Bezeková, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department for Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: j.bezekova@gmail.com.

Miroslav Kročko, Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department for Evaluation and Processing of Animal Products, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: mirokrocko@yahoo.com.