Ann Microbiol (2016) 66:381–387
DOI 10.1007/s13213-015-1120-3

ORIGINAL ARTICLE

# Molecular genetic study of potentially bacteriocinogenic clinical and dairy *Enterococcus* spp. isolates from Bulgaria

**Tanya Strateva**[1] · **Svetoslav G. Dimov**[2] · **Daniela Atanasova**[1] · **Veronika Petkova**[2] · **Encho Savov**[3] · **Ivan Mitov**[1]

Received: 20 May 2015 / Accepted: 11 June 2015 / Published online: 1 July 2015
© Springer-Verlag Berlin Heidelberg and the University of Milan 2015

**Abstract** A total of 424 *Enterococcus* spp. isolates from clinical samples ($n$=398) and traditional Bulgarian artisanal cheeses ($n$=26) were studied in order to evaluate the incidence and expression of bacteriocin genetic determinants. Structural genes coding for enterocin A (*entA*), enterocin P (*entP*), enterocin B (*entB*), enterocin AS-48 (*as-48*) and enterolysin A (*entlA*) were detected by polymerase chain reaction (PCR), while reverse transcription PCR was performed to establish the gene expression. Of the total isolates, 163 (38.4 %) were potentially bacteriocinogenic. The genes typical of *Enterococcus faecium* (*entA*, *entB* and *entP*) were found in 84.0, 8.4 and 15.1 % of the *E. faecium* isolates, respectively. Focusing on *E. faecalis* isolates, the frequencies of *entlA* and *as-48* were 19.3 and 0.7 %, respectively. No significant differences in the gene presence between clinical and dairy isolates were ascertained. Bacteriocin genes were found to be expressed at transcriptional level in 83.4 % of the bacteriocinogenic *Enterococcus* spp. isolates: a single gene in 113 samples (69.3 %), two genes in 21 (12.9 %) and three genes in 2 isolates (1.2 %). The present investigation may serve as a basis for further studies for elucidating the spread of the bacteriocin genetic determinants in the two enterococcal populations (clinical and dairy).

✉ Tanya Strateva
dr.strateva@abv.bg

[1] Department of Medical Microbiology, Faculty of Medicine, Medical University of Sofia, 2 Zdrave Street, 1431 Sofia, Bulgaria

[2] Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

[3] Laboratory of Microbiology, Department of Military Epidemiology and Hygiene, Military Medical Academy, Sofia, Bulgaria

**Keywords** *Enterococcus* spp. · Clinical isolates · Isolates from artisanal cheeses · Bacteriocin-encoding genes · Incidence · Gene expression

## Introduction

Enterococci are lactic acid bacteria (LAB) of significance in medical microbiology, industrial microbiology and public health. They are part of the normal microflora of the gut, urogenital tract and mouth in humans and animals, and are widespread in soil, water, plants and foods (Müller et al. 2001; Giraffa 2002).

Enterococci have traditionally been regarded as low-grade pathogens. Therefore, enterococcal infections could arise as a complication after surgery, invasive procedures, prolonged in-patient stay and precedent antimicrobial therapy or in some hidden rare diseases of the patient (Batistão et al. 2012). During the last two decades, *Enterococcus* spp. (mostly *E. faecalis* and *E. faecium*) have emerged as an increasingly important cause of nosocomial and community-acquired infections, such as urinary tract infections, surgical site and burn wound infections, bacteremia and sepsis, endocarditis, cholecystitis, peritonitis, neonatal meningitis and others (Sood et al. 2008).

Also, enterococci have important implications in the dairy industry. They occur as nonstarter LAB in a variety of cheeses, especially traditional cheeses produced in southern Europe and Asia from raw or pasteurised milk, and in natural milk or whey starter cultures (Franz et al. 2003; Giraffa 2003). Enterococcal strains are involved in ripening of many artisanal cheeses and have also been used as components of cheese starter cultures (Foulquié Moreno et al. 2006). The positive influence of enterococci on cheese seems due to specific biochemical traits such as lipolytic and proteolytic activities, their

capacity to use citrate and pyruvate as carbon sources, and production of aromatic volatile compounds. A few strains are even being promoted as probiotics, contributing to intestinal health by improving the microbial balance of the gut (Domann et al. 2007).

The difference between an enterococcal pathogen and an apparently safe dairy use strain is unclear. It is established that the molecular taxonomy of enterococci does not distinguish these two types of strains (Eaton and Gasson 2001). Many *Enterococcus* spp. strains of clinical and food origin produce antibacterial peptides (bacteriocins), generally called enterocins (Franz et al. 2007). Usually, they are small cationic peptide toxins with bactericidal activity, in most cases against closely related species, which enables microorganisms to occupy their ecological niches. Interest in enterocins has been stimulated by the fact that they are active against Gram-positive dairy-borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and vegetative cells and spores of *Clostridium botulinum* (Franz et al. 2007).

Bacteriocins produced by *Enterococcus* spp. include enterocins A, B and P typical of *E. faecium*, as well as enterocin AS-48 and enterolysin A typical of *E. faecalis*.

The aim of this study was to evaluate the incidence and expression of bacteriocin structural genes among *Enterococcus* spp. strains isolated from clinical specimens and traditional Bulgarian artisanal cheeses.

## Materials and methods

### Clinical isolates

A collection of 398 non-duplicate clinically significant strains of *Enterococcus* spp. (294 *E. faecalis* and 104 *E. faecium*) was investigated. The isolates were recovered between June 2012 and March 2015 from 301 in-patients and 97 outpatients in seven large Bulgarian hospitals. They were obtained from urine ($n$=152), surgical wound or abscesses ($n$=117), genital tract samples ($n$=78), blood ($n$=21), lower respiratory tract samples ($n$=15) and bile ($n$=15).

### Dairy isolates

A total of 26 non-pathogenic *Enterococcus* spp. strains (11 *E. faecalis* and 15 *E. faecium*) were isolated from artisanal cheeses in different mountain regions of Bulgaria. They play an acknowledged role during the ripening.

### Culture media

HiCrome Enterococcus faecium Agar Base (Himedia Labs) was applied for isolation of the *Enterococcus* spp. strains. *E. faecalis* forms blue colonies, while *E. faecium* gives green colonies, surrounded by yellowish colouring of the ambient.

### Biochemical identification with commercial kits and systems

Species identification was done using API Rapid ID 32 Strep (bioMérieux), BBL Crystal Gram-positive ID kit (Becton Dickinson) and the automated system VITEK 2 (bioMérieux).

### DNA isolation

Total DNA from all used strains was isolated with GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich), according to the manufacturer's instructions, from 3-ml overnight cultures inoculated with a single colony.

### Molecular genetic genus and species identification

Polymerase chain reaction (PCR) amplification of the 16S ribosomal ribonucleic acid (rRNA) gene of *Enterococcus* spp. was used for genus identification of all isolates included in this study. The *sodA* genes encoding the enzyme manganese-dependent superoxide dismutase for the most common enterococci were detected with multiplex PCR. *E. faecalis* identification was confirmed by PCR for the *eda1* gene (encoding 2-keto-3-deoxy-6-phosphogluconate aldolase, which is an enzyme taking part into the Entner-Doudoroff pathway and is species-specific for *E. faecalis*). Oligonucleotides used as primers for amplification were synthesized from Alpha DNA (Canada) and are listed in Table 1.

### PCR screening for the presence of bacteriocin genetic determinants

PCR amplification was performed in order to confirm the presence of genes coding for different class bacteriocins according to the current classification system of Franz et al. (2007): class II.1. [enterocin A (*entA*) and enterocin P (*entP*)], class II.3. [enterocin B (*entB*)], class III [enterocin AS-48 (*as-48*)] and class IV [enterolysin A (*entlA*)] bacteriocins. Oligonucleotides used as amplification primers (Alpha DNA) are shown in Table 2.

PCR was carried out with 10 ng of template DNA, 0.25 μM of each primer, 0.2 mM deoxyribonucleoside triphosphates, 1× reaction buffer, 2 mM $MgCl_2$ and 0.5 U Prime *Taq* DNA polymerase (Genet Bio) in a total volume of 25 μl. The DNA was amplified using the following protocol: initial denaturation (94 °C for 5 min), followed by 28–30 cycles of denaturation (94 °C for 35–45 s), annealing (50–60 °C, from 45 s to 1 min) and extension (72 °C, from 45 s to 1 min), with a single final extension of 7 min at 72 °C. PCR products were separated in 1 % agarose gel for 40–60 min at

Table 1 Oligonucleotides used as amplification primers for genus-specific and species-specific identification of *Enterococcus* spp

| Primer pairs[a] | Target gene | Sequence (5′–3′) | Product size (bp) | Annealing temperature (°C) | Source |
|---|---|---|---|---|---|
| E1 | 16S rRNA gene of | TCAACCGGGGAGGGT | 733 | 50 | Deasy et al. 2000 |
| E2 | *Enterococcus* spp. | ATTACTAGCGATTCCGG | | | |
| FL1* | *sodA* of | ACTTATGTGACTAACTTAACC | 360 | 55 | Jackson et al. 2004 |
| FL2* | *E. faecalis* | TAATGGTGAATCTTGGTTTGG | | | |
| FM1* | *sodA* of | GAAAAAACAATAGAAGAATTAT | 215 | 55 | Jackson et al. 2004 |
| FM2* | *E. faecium* | TGCTTTTTTGAATTCTTCTTTA | | | |
| DU1* | *sodA* of | CCTACTGATATTAAGACAGCG | 295 | 55 | Jackson et al. 2004 |
| DU2* | *E. durans* | TAATCCTAAGATAGGTGTTTG | | | |
| EDA1_F1 | *eda1*of | GGGGACAGTTTTGGATGCTA | 404 | 60 | Peykov et al. 2012 |
| EDA1_R1 | *E. faecalis* | TCCATATAGGCTTGGGCAAC | | | |

*rRNA* ribosomal ribonucleic acid; *sodA* manganese-dependent superoxide dismutase-encoding gene; *eda1* gene for 2-keto-3-deoxy-6-phosphogluconate aldolase (an enzyme taking part into the Entner-Doudoroff pathway, which is specific for *E. faecalis*)

[a] The three pairs marked with asterisks are used in a multiplex polymerase chain reaction

140 V, stained with ethidium bromide (0.5 μg/ml) and detected by UV transillumination (wavelength 312 nm). Amplified genes were identified on the basis of fragment length presented in Tables 1 and 2.

## Reverse transcription PCR

Reverse transcription PCR (RT-PCR) was performed to ascertain the bacteriocin gene expression at transcriptional level. Total bacterial RNA was isolated with GeneJET RNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. The purified total RNA was used immediately or frozen quickly in liquid nitrogen and conserved at −80 °C. Immediately before the reverse transcription reaction, the RNA was treated with DNAse I (Thermo Scientific) by adding 1 μl of DNAse buffer (with $MgCl_2$ and 1 μl of DNAse I) to 8 μl of the RNA samples, followed by incubation for 30 min at 37 °C. The reaction was stopped by inactivating the enzyme by adding 1 μl of the provided EDTA solution and heating to 65 °C for 10 min, followed by chilling on ice. RevertAid Reverse Transcriptase kit (Thermo Scientific) was used for synthesis of the first-strand cDNA. The reaction was carried out with 4 μl of the reaction buffer, 2 μl of the bacteriocin-specific primer (at 10 μM concentration), 2 μl of the provided dNTPs mix, 1 μl M-MuLV reverse transcriptase, 1 μl RiboLock RNAse inhibitor and 8 μl of di$H_2O$ in a total volume of 20 μl for 1 h at 37 °C. The reverse transcription was stopped by incubating the reaction mixture for 5 min at 70 °C, followed by chilling on ice. The obtained single-strand cDNAs were further used as matrices for PCR reactions, which were carried out in a MJ Research PTC-200 Thermal Cycler under the same conditions as the standard PCR amplifications for bacteriocin genetic determinants.

## Statistical analysis

The distribution of bacteriocin structural genes with respect to isolate origin was compared using the Student's *t* test. A *P* value below 0.05 was considered to be statistically significant.

Table 2 Oligonucleotides used as primers for amplification of bacteriocin structural genes in *Enterococcus* spp

| Primer pairs | Target gene | Sequence (5′–3′) | Product size (bp) | Annealing temperature (°C) | Source |
|---|---|---|---|---|---|
| EntlA3 | *entlA* | GGACAACAATTCGGGAACACT | 1007 | 59 | Nigutova et al. 2007 |
| EntlA9 | | GCCAAGTAAAGGTAGAATAAA | | | |
| As48-1 | *as-48* | AATAAACTACATGGGT | 377 | 50 | Fernández et al. 2007 |
| As48-5 | | CCAAGCAATAACTGCTCTTT | | | |
| EntA(f) | *entA* | AAATATTATGGAAATGGAGTGTAT | 125 | 60 | du Toit et al. 2000 |
| EntA(r) | | GCACTTCCCTGGAATTGCTC | | | |
| EntB(f) | *entB* | GAAAATGATCACAGAATGCCTA | 160 | 60 | du Toit et al. 2000 |
| EntB(r) | | GTTGCATTTAGAGTATACATTTG | | | |
| EntP1 | *entP* | ATGAGAAAAAAATTATTTAGTTT | 215 | 60 | Gutiérrez et al. 2005 |
| EntP2 | | TTAATGTCCCATACCTGCCAAACC | | | |

*entlA* gene for enterolysin A of *E. faecalis*; *as-48* gene for enterocin AS-48 of *E. faecalis*; *entA* gene for enterocin A of *E. faecium*; *entB* gene for enterocin B of *E. fecium*; *entP* gene for enterocin P of *E. faecium*

## Results

### Incidence of bacteriocin structural genes among all studied *Enterococcus* spp. isolates

A total of 163 of the 424 studied enterococcal isolates (38.4 %) were potentially bacteriocinogenic, according to the PCR screening. The genes typical of *E. faecium* (*entA*, *entB* and *entP*) were found in 84.0, 8.4 and 15.1 % of the *E. faecium* isolates, respectively. Focusing on *E. faecalis* isolates, the frequencies of *entlA* and *as-48* were 19.3 and 0.7 %, respectively. The structural gene for enterocin A was widely distributed among *E. faecium* isolates included in this study, whereas that of enterocin B generally (70 %) occurred in the presence of *entA*.

Figures 1 and 2 show the electrophoretograms for four bacteriocin-encoding genes for which amplification products were obtained (*entlA*, *as-48*, *entA* and *entB*).

### Comparative frequency of bacteriocin structural genes among *Enterococcus* spp. isolates from clinical samples and artisanal cheeses

The bacteriocin gene incidence in the two different collections (clinical, pathogenic and dairy, non-pathogenic *Enterococcus* spp. isolates) is presented in Table 3, in which, noticeably, is the more widespread dissemination of genes typical of *E. faecium* (*entA*, *entB* and *entP*) among cheese isolates than that in clinical isolates, whereas the genes of *E. faecalis* (*entlA* and *as-48*) were found more frequently in the pathogenic enterococci. No significant differences in the gene presence between clinical and dairy isolates were ascertained. Four of our cheese *E. faecium* isolates carried the enterocin A and B structural genes (*entA* and *entB*) simultaneously, while another four isolates carried the combination of genes *entA*+*entP*.

### Expression of bacteriocin structural genes among the bacteriocinogenic *Enterococcus* spp. isolates

Bacteriocin genes were found to be expressed at transcriptional level in 83.4 % ($n$=136) of the 163 potentially bacteriocinogenic isolates *Enterococcus* spp. isolates: a single gene in 113 samples (69.3 %), two genes in 21 (12.9 %) and three genes in 2 isolates (1.2 %). No gene expression appeared in 16.5 % ($n$=27) of the *Enterococcus* spp. strains presenting bacteriocin genes (Fig. 3). The most frequent co-expressed genes were *entA* and *entP* (8 % of the potentially bacteriocinogenic strains), followed by *entA*+*entB* (3.7 %). Intriguingly, two isolates (1.2 %) expressed three genes simultaneously, *entA*+*entB*+*entP*.

## Discussion

Enterococci are usually isolated from fermented foods, in which they contribute to the ripening/organoleptic characteristics (Giraffa 2003), but nowadays are considered emerging pathogens, due to an increase of antibiotic resistances and production of virulence traits Morrison et al. 1997). Both groups, GRAS (generally recognized as safe) *Enterococcus* spp. strains and clinically significant *Enterococcus* spp. strains, have a potential to produce different bacteriocins, some of which have been well characterized, e.g., enterocins A, B, P, AS-48, enterolysin A, etc.

In the present study, the most commonly occurring bacteriocin-encoding gene was the *entA*. Similar to our results, the *entA* was the most widespread gene among *E. faecium* isolates of different sources, whereas the *entB* was always associated with the presence of *entA*, as referred to in other investigations (De Vuyst et al. 2003; Poeta et al. 2007; Sabia et al. 2008). Enterocin B is a broad-spectrum bacteriocin of the class II.2. (Franz et al. 2007), that acts synergistically with enterocin A, a member of the

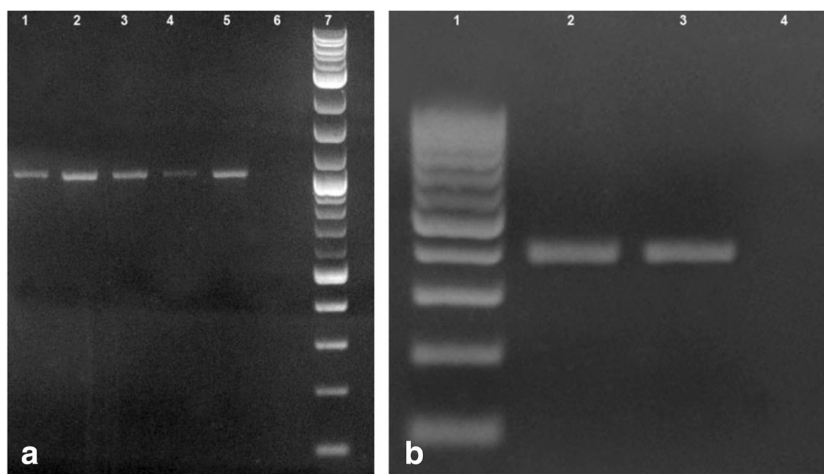


**Fig. 1** Amplification results for genes encoding entrolysin A (**a**) and enterocin AS-48 (**b**) in *E. faecalis*. **a** Lines *1–5*, specific amplification products corresponded to the *entlA* gene (1007 bp); line *6*, (−) negative control; line *7*, 100-bp Ladder. **b** Line *1*, 100-bp Ladder; lines *2*, *3*, specific amplification products corresponded to the *as-48* gene (377 bp); line *4*, (−) negative control

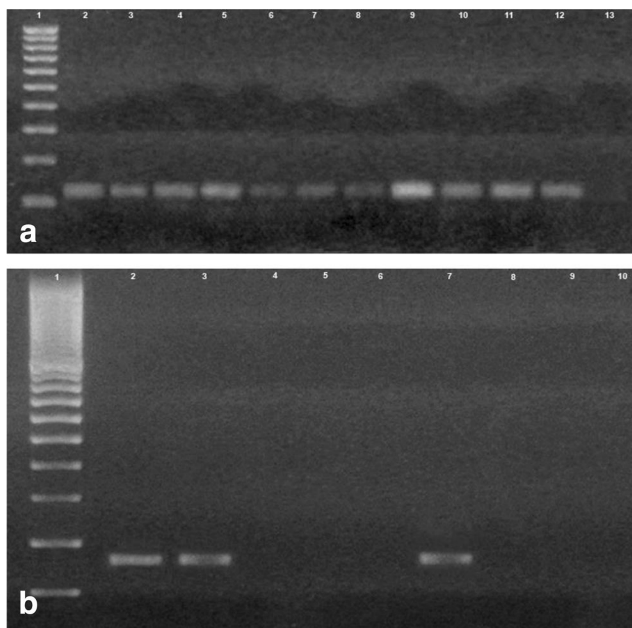


**Fig. 2** Amplification results for genes encoding enterocin A (**a**) and enterocin B (**b**) in *E. faecium*. **a** Line *1*, 100-bp Ladder; lines *2–12*, specific amplification products corresponded to the *entA* gene (125 bp); line *13*, (−) negative control. **b** Line *1*, 100-bp Ladder; lines *2*, *3*, *7*, specific amplification products corresponded to the *entB* gene (160 bp); lines *4–6*, *8–9*, (−) negative PCR products; line *10*, (−) negative control

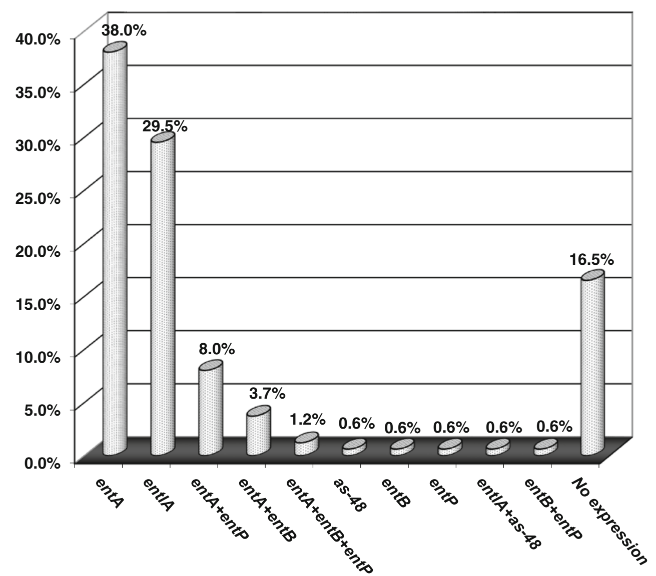


**Fig. 3** Expression of bacteriocin structural genes (as percentages) among the potentially bacteriocinogenic *Enterococcus* spp. isolates ($n$=163)

pediocin family (Casaus et al. 1997). We established significantly higher prevalence of spread of *entA* among *E. faecium* isolates compared to that in vancomycin-resistant enterococci collected from clinical, dairy and animal samples in Italy (84.0 vs. 46.1 %, $p<0.01$) (Sabia et al. 2008). In the Italian investigation mentioned above, *entP* and *as-48* were not detected in vancomycin-resistant *E. faecium* and *E. faecalis* isolates, respectively.

Recently, Padilla et al. (2012) studied the presence of some bacteriocin determinants in clinical *E. faecalis* strains isolated in Chile during the period 2008–2009. Contrary to our results, they found significantly higher frequency for *entlA* (31.3 vs 19.7 %, $p<0.01$) and *as-48* (9.3 % / 0.7 %, $p<0.02$). Moreover, they proved higher rates of incidence of these genes among isolates causing bacteremia and urinary tract infections (UTIs) compared to our analogous isolates: *entlA*

**Table 3** PCR detection of bacteriocin structural genes among 424 *Enterococcus* spp. isolates from different sources

| Gene | Number (%) of isolates with the respective gene | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Urine ($N_{FL}$=105) ($N_{FM}$=47) | Wounds ($N_{FL}$=82) ($N_{FM}$=35) | Genital tract [a] ($N_{FL}$=74) ($N_{FM}$=4) | Blood ($N_{FL}$=14) ($N_{FM}$=7) | Respiratory tract [b] ($N_{FL}$=10) ($N_{FM}$=5) | Bile ($N_{FL}$=9) ($N_{FM}$=6) | All clinical specimens ($N_{FL}$=294) ($N_{FM}$=104) | Cheese ($N_{FL}$=11) ($N_{FM}$=15) | Total ($N_{FL}$=305) ($N_{FM}$=119) |
| *entlA* FL | 20 (19.0) | 10 (12.2) | 18 (24.3) | 4 (28.6) | 4 (40.0) | 2 (22.2) | 58 (19.7) | 1 (9.1 %) | 59 (19.3) |
| *entlA* FM | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| *as-48* FL | 1 (1.0) | 0 (0) | 1 (1.4) | 0 (0) | 0 (0) | 0 (0) | 2 (0.7) | 0 (0) | 2 (0.7) |
| *as-48* FM | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| *entA* FL | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| *entA* FM | 39 (83.0) | 27 (77.1) | 4 (100) | 6 (85.7) | 4 (80.0) | 6 (100) | 86 (82.7) | 14 (93.3) | 100 (84.0) |
| *entB* FL | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| *entB* FM | 2 (4.3) | 4 (11.4) | 0 (0) | (0) | 0 (0) | 0 (0) | 6 (5.8) | 4 (26.7) | 10 (8.4) |
| *entP* FL | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| *entP* FM | 8 (17.0) | 5 (14.3) | 1 (25.0) | 0 (0) | 0 (0) | 0 (0) | 14 (13.5) | 4 (26.7) | 18 (15.1) |

*FL*, *E. faecalis*; *FM*, *E. faecium*; *N/A* not applicable; *entlA* gene for enterolysin A of *E. faecalis*; *as-48* gene for enterocin AS-48 of *E. faecalis*; *entA* gene for enterocin A of *E. faecium*; *entB* gene for enterocin B of *E. fecium*; *entP* gene for enterocin P of *E. faecium*

[a] Genital tract specimens: sperm, urethral, vaginal and cervical samples

[b] Respiratory tract: sputum and tracheobronchial aspirates

for blood isolates – 42 % in Chile versus 28.6 % in our study; *entlA*, UTIs isolates – 30 %/19 %; *as-48*, blood isolates – 42 %/0 %, $p<0.001$; *as-48*, UTIs isolates - 30 %/1 %, $p<0.001$.

Previous studies showed that PCR detection of more than one bacteriocin-encoding gene in the same *Enterococcus* spp. isolate from artisanal cheeses is not unusual (Edalatian et al. 2012; Özden Tuncer et al. 2013). In the present study, there was a higher dissemination rate of *entA* among *E. faecium* cheese isolates (93.3 %) compared to that among *E. faecium* isolated from traditional Iranian raw milk cheeses (72.7 %), whereas the *entB* and *entP* revealed lower frequency rates in our isolates than among Iranian ones (26.7 vs. 54.5 %) (Edalatian et al. 2012). Recently, Özden Tuncer et al. (2013) isolated three bacteriocin-producer strains of *E. faecium* from artisanal Turkish tulum cheese and established the occurrence of eneterocin genes. All these strains bore the *entA* and *entB* genes, while two strains also contained the *entP* gene.

In our study, we chose to perform simple RT-PCR reactions to monitor solely whether or not the bacteriocins were expressed at RNA level. Despite the fact that, nowadays, multiplex quantitative PCR (qPCR) has been applied as a technique that allows the levels of individual enterocin gene expression to be monitored and determination of how expression is altered under different growth conditions (Williams and Chanos 2012), for the purposes of the present investigation this was not necessary due to the fact that the levels of bacteriocin production is often strongly influenced by the environmental conditions, such as temperature, pH, salts concentrations, presence of diverse sugars, etc. (Dimov et al. 2008; Raykova et al. 2008; Peykov et al. 2008). This means the exact quantitation of the levels of bacteriocin expression in controlled cultivation conditions would surely differ from the growth conditions of the strains' natural environments, clinical or dairy. On the other hand, simple RT-PCR is sensitive enough to detect even minimal expression levels, thus avoiding to perform relatively more expensive and time-consuming RT-qPCR, which, even if it was carried out, would give us erroneous results.

In conclusion, more than one-third of the isolates included in the present study were revealed to be bacteriocinogenic. To our knowledge, this is the first investigation in Bulgaria for the incidence of bacteriocin-encoding genes among clinically relevant and non-pathogenic enterococci, which may serve as a basis for additional studies for elucidating the spread of the bacteriocin genetic determinants among the two different types of enterococcal populations.

*Enterococcus* spp. strains that produce bacteriocins are of increasing interest for science and industry because they seem to have an ecological advantage when compared with other nonproducing bacteria which inhabit the same ecosystem or which concur for colonization and for invasion in a particular ecological niche (Poeta et al. 2007). In addition, enterococci are good acidifiers in the presence of available sugars, and the inhibitory effect due to acid production could be considered an advantage in addition to bacteriocin production, and is equally responsible for survival and colonization (Sabia et al. 2008).

**Acknowledgment** This work was supported by a grant from the Medical University of Sofia (Council of Medical Science, project no. 6/2014, grant no. 11/2014).

## References

Batistão DW, Gontijo-Filho PP, Conceição N, Oliveira AG (2012) Risk factors for vancomycin-resistant enterococci colonisation in critically ill patients. Mem Inst Oswaldo Cruz 107:57–63. doi:10.1590/S0074-02762012000100008

Casaus P, Nilsen T, Cintas LM, Nes IF, Hernandez PE, Holo H (1997) Enterocin B, a new bacteriocin from *Enterococcus faecium* T136 which can act synergistically with enterocin A. Microbiology 143:2287–2294. doi:10.1099/00221287-143-7-2287

De Vuyst L, Foulquie Moreno MR, Reverts H (2003) Screening for enterocins and detection of hemolysin and vancomycin resistance in enterococci of different origins. Int J Dairy Microbiol 84:299–318. doi:10.1016/S0168-1605(02)00425-7

Deasy BM, Rea MC, Fitzgerald GF, Cogan TM, Beresford TP (2000) A rapid PCR based method to distinguish between *Lactococcus* and *Enterococcus*. Syst Appl Microbiol 23:510–522. doi:10.1016/S0723-2020(00)80025-9

Dimov SG, Peykov SZ, Raykova DS, Ivanova PM (2008) Influence of diverse sugars on BLIS production by three different *Enterococcus* strains. Trakia J Sci 6(1):54–59

Domann E, Hain T, Ghai R, Billion A, Kuenne C, Zimmermann K, Chakraborty T (2007) Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1. Int J Med Microbiol 297:533–539. doi:10.1016/j.ijmm.2007.02.008

du Toit M, Franz CM, Dicks LM, Holzapfel WH (2000) Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. J Appl Microbiol 88:482–494. doi:10.1046/j.1365-2672.2000.00986.x

Eaton TJ, Gasson MJ (2001) Molecular screening of *Enterococcus* virulence determinants and potential for genetic exchange between dairy and medical isolates. Appl Environ Microbiol 67:1628–1635. doi:10.1128/AEM.67.4.1628-1635.2001

Edalatian MR, Habibi Najafi MB, Mortazavi SA, Alegría A, Delgrado S, Bassami MR, Mayo B (2012) Production of bacteriocins by *Enterococcus* spp. isolated from traditional, Iranian, raw milk cheeses, and detection of their encoding genes. Eur Dairy Res Technol 234:789–797. doi:10.1007/s00217-012-1697-8

Fernández M, Martínez-Bueno M, Martín MC, Valdivia E, Maqueda M (2007) Heterologous expression of enterocin AS-48 in several strains of lactic acid bacteria. J Appl Microbiol 102:1350–1361. doi:10.1111/j.1365-2672.2006.03194.x

Foulquié Moreno MR, Sarantinopoulos P, Tsakidou E, De Vuyst L (2006) The role and application of enterococci in food and health. Int J Food Microbiol 106:1–24. doi:10.1016/j.ijfoodmicro.2005.06.026

Franz CMAP, Stiles ME, Schleifer KH, Holzapfel WH (2003) Enterococci in foods – a conundrum for food safety. Int J Food Microbiol 88:105–122. doi:10.1016/S0168-1605(03)00174-0

Franz CMAP, van Belkum MJ, Holzapfel WH, Abriouel H, Gálvez A (2007) Diversity of enterococcal bacteriocins and their grouping in a

new classification scheme. FEMS Microbiol Rev 31:293–310. doi:10.1111/j.1574-6976.2007.00064.x

Giraffa G (2002) Enterococci from foods. FEMS Microbiol Rev 26:163–171. doi:10.1111/j.1574-6976.2002.tb00608.x

Giraffa G (2003) Functionality of enterococci in dairy products. Int J Dairy Microbiol 88:215–222. doi:10.1016/S0168-1605(03)00183-1

Gutiérrez J, Criado R, Citti R, Martín M, Herranz C, Nes IF, Cintas LM, Hernández PE (2005) Cloning, production and functional expression of enterocin P, a sec-dependent bacteriocin produced by *Enterococcus faecium* P13, in *Escherichia coli*. Int J Dairy Microbiol 103:239–250. doi:10.1016/j.ijfoodmicro.2004.11.035

Jackson CR, Fedorka-Cray PJ, Barrett JB (2004) Use of a genus- and species-specific multiplex PCR for identification of enterococci. J Clin Microbiol 42:3558–3565. doi:10.1128/JCM.42.8.3558-3565.2004

Morrison D, Woodford N, Cookson B (1997) Enterococci as emerging pathogens of humans. Soc Appl Bacteriol Symp Ser 26:89S–99S

Müller T, Ulrich A, Ott EM, Müller M (2001) Identification of plant-associated enterococci. J Appl Microbiol 91:268–278. doi:10.1046/j.1365-2672.2001.01373.x

Nigutova K, Morovsky M, Pristas P, Teather RM, Holo H, Javorsky P (2007) Production of enterolysin A by rumen *Enterococcus faecalis* strain and occurrence of enlA homologues among ruminal Gram-positive cocci. J Appl Microbiol 102:563–569. doi:10.1111/j.1365-2672.2006.03068.x

Özden Tuncer B, Ay Z, Tuncer Y (2013) Occurrence of enterocin genes, virulence factors, and antibiotic resistance in 3 bacteriocin-producer *Enterococcus faecium* strains isolated from Turkish tulum cheese. Turk J Biol 37:443–449. doi:10.3906/biy-1209-26

Padilla EC, Núñez MA, Padilla GA, Lobos GO (2012) Virulence genes and bacteriocins in *Enterococcus faecalis* strains isolated from different clinical samples in Maule Region, Chile. Rev Chil Infect 29:55–61. doi:10.4067/S0716-10182012000100010, article in Spanish with an abstract in English

Peykov SZ, Raykova DS, Dimov SG (2008) NaCl suppression of bacteriocin production by three *Enterococcus* strains. Trakia J Sci 6(1):45–48

Peykov SZ, Aleksandrova VD, Dimov SG (2012) Rapid identification of *Enterococcus faecalis* by species-specific primers based on the genes involved in the Entner-Doudoroff pathway. Mol Biol Rep 39:7025–7030. doi:10.1007/s11033-012-1533-z

Poeta P, Costa D, Rojo-Bezares B, Zarazaga M, Klibi N, Rodrigues J, Torres C (2007) Detection of antimicrobial activities and bacteriocin structural genes in faecal enterococci of wild animals. Microbiol Res 162:257–263. doi:10.1016/j.micres.2006.06.003

Raykova DS, Peykov SZ, Dimov SG (2008) Growth kinetics and bacteriocin production by three *Enterococcus* strains under low-temperature stress. Trakia J Sci 6(1):49–53

Sabia C, de Niederhäusern S, Guerrieri E, Messi P, Anacarso I, Manicardi G, Bondi M (2008) Detection of bacteriocin production and virulence traits in vancomycin-resistant enterococci of different sources. J Appl Microbiol 104:970–979. doi:10.1111/j.1365-2672.2007.03612.x

Sood S, Malhorta M, Das BK, Kapil A (2008) Enterococcal infections and antimicrobial resistance. Indian J Med Res 128:111–121

Williams DR, Chanos P (2012) Application of anti-listerial bacteriocins: monitoring enterocin expression by multiplex relative reverse transcription-PCR. Biochem Soc Transact 40:1544–1548. doi:10.1042/BST20120191