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Bacteriocin-Producing Enterococci from Rabbit Meat

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ABSTRACT

Aims: Enterococci are lactic acid bacteria belonging to the division *Firmicutes*. They occur in different ecosystems, rabbits including. Enterococci can possess probiotic properties and produce antimicrobial substances-bacteriocins. Rabbit meat as nutritionally healthy food offers novel source to study bacteriocin-producing and/or probiotic enterococci.
Methology and result: Enterococci were detected from rabbit meat samples (42). Most of the isolates were allotted to the species *Enterococcus faecium* by PCR method. The isolates have possessed the structural genes for enterocins A, P, B production. The inhibitory substances produced by the isolated enterococci inhibited the growth of 12 indicators. Of 34 isolates, 15 strains have shown the antimicrobial activity against *L. monocytogenes* CCM 4699, 12 strains against *S. aureus* 3A3, 10 strains against *S. aureus* 5A2 as well as *Salmonella enterica* serovar Enteritidis PT4. Moreover, enterococci have tolerated 5 % bile, low pH; they have produced lactid acid in the amount from 0.740 ± 0.091 to 1.720 ± 0.095 mmol/l. The isolates were mostly sensitive to antibiotics.
Conclusion, significance and impact of study: Bacteriocin-producing strain *E. faecium* M3a has been selected for more detail characterization of its bacteriocin and probiotic properties with the aim for its further application as an additive.

Keywords: bacteriocin, probiotic bacteria, antimicrobial effect

INTRODUCTION

Enterococci are Gram-positive, facultative anaerobic, catalase-negative lactic acid bacteria (LAB) taxonomically allotted to the division Firmicutes, the class Bacilli, the order Lactobacillales, the family Enterococcacae and the genus Enterococcus (Bergeys Manual of Systematic Bacteriology, 2009). They have been isolated from different ecosystems, rabbit meat including (Devriese *et al.*, 1993; Aymerich *et al.*, 1996; Franz *et al.*, 2007; Szabóová, 2011). In general, enterococci are studied for their probiotic and bacteriocin-producing properties to have beneficial effect *e.g.* as novel additives in animal nutrition. Rabbit meat is considered to be one of the healthiest meats because of its easily digestion and dietetic properties, *e.g*. high values of proteins (20-21 %), unsaturated fatty acids, potassium, phosphorus, magnesium and low fat, cholesterol, sodium contents (Dalle Zotte, 2002).

The occurrence of enterococci in rabbit meat could be mostly originated from the environment in the time of slaughtering. There, the majority isolates have been allotted to the species *Enterococcus faecium* (Szaboová, 2011). The ability of LAB to produce natural antimicrobial substances - organic acids, diacetyl, hydrogen peroxide and bacteriocins has been well known (Franz *et al.*, 2007). Bacteriocins produced by enterococcci, mostly enterocins are of considerable interest because they have provided an inhibitory activity against spoilage microbiota such as *Listeria monocytogenes, Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Enteritidis (Lauková and Czikková, 1998, 1999; Franz *et al.*, 2007; Levkut *et al*., 2009). Effect of several bacteriocins produced by enterococci, mainly those produced by the strians of the species *Enterococcus faecium* of different origin have been previously reported and they have been more frequently applied as protective cultures or feed supplements (Strompfová *et al.*, 2003, 2006; Audisio *et al.* 1999; Szabóová *et al.*, 2008; Pogány Simonová *et al.*, 2009, Levkut *et al.,* 2011). Taking into the principal interest the health property of rabbit meat, the novelty point of this basic research has been focused on enterococci isolated from rabbit meat to study their bacteriocin activity and some probiotic properties (which has not been studied yet).

MATERIALS AND METHODS

Isolation and Identification of Bacteria

Enterococci were isolated from rabbit back limb (*musculus biceps femoris*) of 42 rabbits (males, Hyplus breed at the farm of Animal Production Research Centre, Nitra, Slovakia). Animal handling and sampling was provided according to the guidelines stated in the Guide for the

Care and Use of Laboratory Animals accepted by Slovak Governmental Veterinary Institution and Ethic Commission. The samples (10 g) were treated by the standard microbiological method according to ISO using appropriate dilutions in Buffered Peptone Water (90 ml, Biomark, India). The appropriate dilutions were plated onto Kanamycin Esculin Agar (Biomark, India), incubated at 37 ºC for 24 h in partially CO2/air atmosphere.

The counts of enterococci were expressed in the colony forming units (cfu) and quoted as means ± SD. Colonies of enterococci (34) were randomly picked up, checked for purity and maintained on Kanamycin Esculin Agar. The isolates were genotyped using the polymerase chain reaction-PCR method (Techgene, KRD Thermocycler-Techne, The United Kingdom) followed by the agarose electrophoresis in 0.8% agarose gels (Sigma, Germany) buffered with 1xTAE (Merck) containing 1 µg/ml of ethidium bromide (Sigma). The molecular mass standard (Promega, USA) was used according to the manufacturers' instructions. DNA (template) from each strain was isolated by the rapid alkaline lysis method (Baele *et al*. 2000). Ten microlitres of the template was added to 39.5 µl of the reagent mixture which contained 0.5 µM each of the primers; 0.2 mM each of the deoxynucleotides (dATP, dTTP, dCTP, dGTP) – dNTPs (Invitrogen); 2.5 mM of MgCl2 (Invitrogen); 10xPCR buffer (Invitrogen); 1.25 U of Taq polymerase (Invitrogen) and H20 to the total volume of 50 µl.

The sequences of the primer pairs prepared according to Woodford *et al.* (1997) and used for DNA amplification of *Enterococcus faecium* were as follows: 5´-GCAAGCTTCTTAGAGA-3´ and 5´-CATCGTGTAAGCTA ACTTC-3´ (Invitrogen). The sequences of the primer pairs for DNA amplification of *Enterococcus faecalis* were 5´-ATCAAGTACAGTTAGTCTT-3´ and 5´-ACGATTCAAAGC TAACTG-3´ (Invitrogen). The amplification protocol was as follows: initial denaturation at 95 °C for 2 min, 40 cycles of 95 °C for 1 min, 55 ºC for 1 min, 72 °C for 1 min, 72 ºC for 10 min. *E. faecium* EK13 (CCM 7419, our isolate and *E. faecalis* CCM 4224 (Czech Culture Collection, Brno, Czech Republic) were used as positive controls.

Detection of The Structural Genes for Bacteriocin Production

The strains were tested to possess the following enterocins (*Ent*) genes: *Ent* A, B, P and L50B. Enterocins mentioned, have been detected most frequently in different enterococcal strains (Strompfová and Lauková, 2007, 2009; Lauková *et al.*, 2008a, 2008b).DNA was extracted by rapid alkaline lysis method (Baele *et al.*, 2000). Template (2 µl) was added to 8.75 µl of the reagent mixture which contained 0.5 µl of each primer, 1 µl of (10 nmol/L) dNTPs (Invitrogen), 1.5 µl of (5 mmol/L) $MgCl_2$ (Invitrogen), 5 µl of 10x reaction buffer (Invitrogen), 0.25 µl of 1 U Taq polymerase (Invitrogen) and water to a total volume of 50 µl. The sequences of the primers pairs used for PCR amplification of the enterocin structural genes - *Ent* A, P, L50B and B are summarized in **Table 1**. The reaction conditions for *Ent* A detection included 5 min denaturation at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 58 °C, 30 s at 72 °C; then 5 min at 72 °C and a cool down to 4 °C. For *Ent* P, L50B and B, the temperature 56 °C instead of 58 °C was used as the annealing temperature. PCR products were visualised by 2% agarose electrophoresis, containing 1 µg of ethidium bromide. Positive control strains were: *E. faecium* EK13 (Mareková *et al.,* 2003) for *Ent* A; *E. faecium* AL41 (Lauková *et al.*, 2003) for *Ent* P; *E. faecium* L50 (Cintas *et al.*, 1998) for *Ent* L50B, B.

Bacteriocin Activity

Bacteriocin activity was tested by the qualitative agar-diffusion technique according to Skalka *et al.* (1983) using BHI agar. The target of the indicator bacteria used have represented rabbits isolates as well as isolates depended on the phylogenetic relation with enterococci *e.g*. listeriae. The principal indicator strain was *Enterococcus avium* EA5 (our isolate from faeces of piglet), *E. faecium* CCM 7420 (our isolate from rabbits faeces deponed to Czech Culture Collection of Microorganisms in Brno-CCM, Czech Republic), *Listeria innocua* LMG 13568 (Collection of Microorganisms at University of Ghent, Belgium), *L. monocytogenes* CCM 4699 (clinical isolate, CCM), *L. monocytogenes 2024, L. monocytogenes* 7223*, L. monocytogenes* 7562 (isolates from food, Veterinary Institute, Olomouc, Czech Republic), *Salmonella enterica* serovar Enteritidis PT4 (Dr. Šišák, Brno, Czech Republic), *Staphylococcus aureus* SA5 (our isolate from mastitis milk), *S. aureus* 3A2, 3A3, 5A2 (our isolates from rabbits faeces). The inhibitory activity was expressed in mm.

Resistance to Bile, Low pH, Lactic Acid Production, Sensitivity/Resistance to Antimicrobials

The resistance to bile was tested according to Gilliland and Walker (1990). Brain Heart Infusion broth (BHI, Becton and Dickinson, USA) was prepared by the addition of 5 % (w/v) oxgall (Becton and Dickinson). The volume 50 µl of an 18 h culture of each strain was added to 5 ml of BHI broth with oxgall. After incubation at 37 °C for 24 h, the bacterial growth of strains was measured using a spectrophotometer (Specol 11, Jena, Germany) at 600 nm. Viable cells were estimated at 0 h and after 24 h of the incubation on M-Enterococcus agar (ME agar, Becton and Dickinson, Cockeysville, USA).

The resistance to low pH (3.0) was tested according to Jin *et al.* (1998). The cells of overnight cultures in BHI (Becton and Dickinson) were harvested by centrifugation (2 000 x *g* for 15 min), resuspended in 0.05 M phosphate buffer of pH 3.0 adjusted with 1 *N* HCl and kept at 37 °C for 1, 2 and 3 h. The cfu were determined on ME agar (Becton and Dickinson). Lactic acid was measured according to Pryce (1969), expressed in mmol/l and the lactic acid values were quoted as means ± standard deviation (SD). Sensitivity/resistance to antimicrobials

Table 1: The sequences of the primer pairs (F- forward; R- reverse) used for PCR- amplification of the structural genes of enterocins *Ent* A, P, L 50B, B.

*Ent*A	F 5´ -GGT ACC ACT CAT AGT GC AAA-3´ R 5´-CCC TGG AAT TGC TCC ACC TAA-3´	Aymerich *et al*., 1996
*Ent*P	F 5´-GCT ACG CGT TCA TAT GGT AAT-3´ R 5´-TCC TGC AAT ATT CTC TTT AGC-3´	Cintas *et al.*, 1997
*Ent*L50B	F 5´-ATG GGA GCA ATC GCA AAA TTA-3´ R 5´-TAG CCA TTT TTC AAT TTG ATC-3´	Cintas *et al*., 1998
*Ent*B	F 5´-CAA AAT GTA AAA GAA TTA AGA TCG-3´ R 5´-AGA GTA TAC ATT TGC TAA CCC-3´	Casaus *et al*., 1997

Table 2: Genotypization, detection of the structural genes for enterocin (*Ent*) production, lactic acid production (LA) by rabbit meat enterococci

	Genotypization		Detection of structural genes				LA production
	E.faecium	*E.faecalis*	*Ent* A	*Ent* P	*Ent* B	*Ent* L50B	(mmol/l)
M1C	+	-	-	-	+	-	0.740±0.091
M2C	+	-	-	-	+	-	0.750±0.065
M7C	+	-	-	+	+	-	1.380±0.011
M7b	+	-	-	-	-	-	1.290±0.042
M4C	+	-	-	-	-	-	1.240±0.053
M6C	+	-	-	-	-	-	1.290±0.030
M5A	+	-	-	-	-	-	1.480±0.215
M3b	+	-	-	-	-	-	1.470±0.024
M1b	+	-	-	-	-	-	1.160±0.059
M2A	+	-	-	-	-	-	1.040±0.039
M2cA	+	-	-	-	-	-	1.270±0.128
M2cB	+	-	-	-	-	-	1.200±0.047
M3a	+	-	+	-	+	-	1.430±0.003
M4aA	+	-	-	-	-	-	1.210±0.074
M4aB	-	-	-	-	-	-	1.380±0.051
M5aA	-	-	-	-	-	-	1.260±0.018
M5aB	-	-	-	-	-	-	1.170±0.062
M6b	-	-	-	-	-	-	1.140±0.101
M7bA	-	-	-	-	-	-	1.340±0.181
M7bB	-	-	-	-	-	-	1.120±0.242
1 BM	-	-	-	-	-	-	1.460±0.254
3 AM	-	-	+	-	-	-	1.570±0.033
4BM1	+	-	-	-	-	-	1.470±0.023
4BM2	-	-	-	-	-	-	1.540±0.162
5BM1	+	-	-	-	-	-	1.200±0.053
5BM2	-	-	-	-	-	-	1.700±0.013
M1B	+	-	-	-	-	-	1.460±0.148
M2c	-	-	-	-	-	-	0.880±0.021
M1c	+	-	-	-	-	-	1.710±0.019
M5a	-	-	-	-	-	-	1.720±0.095
M6c	+	-	-	-	-	-	1.050±0.079
M3A	-	-	-	-	-	-	1.310±0.032
M4B	+	-	-	-	-	-	1.56 ± 0.226
M2a	-	-	-	-	-	-	1.130±0.098

was tested by the agar disk diffusion method (NCCLS 2002) on BHI agar. The following antibiotic disks were used: streptomycin (30 µg; LaChema, Czech Republic), neomycin (5 µg), gentamicin, ampicillin (10 µg), erythromycin (15 µg), kanamycin, tetracycline, vancomycin, rifampicin, chloramphenicol (30 µg, Becton and Dickinson). After incubation at 37 °C for 18 h, the strains were classified as resistant or sensitive according to the manufacturers´ instructions and the size of the inhibitory zones was expressed in mm.

RESULTS

The counts of enterococci have ranged from 10^1 to10^2 cfu/g. Among 34 isolates, 67.6 % strains have been allotted to the species *E. faecium* and 32.4 % isolate for *Ent* A, B, P production have been detected in the isolated strains (**Table 2**). No strain has possessed gene for *Ent* L50B. Among 34 isolates, in 29 strains (85.3 %) no s were not allotted to the species yet. The structural genes genes (from the genes tested) have been detected. In *E*.

Table 3: Resistance of enterococci to antimicrobials[a]

Strains	Rif[a]	Kan[a]	Str[a]	Ery[a]	Ttc[a]	Gen[a]	Van[a]	Chc[a]	Amp[a]	Neo[a]
Enterococci	tested strains/resistant strains									
n=34	34/0	34/33	34/6	34/4	34/15	34/12	34/21	34/1	34/21	34/27
R in %	0.0	97.0	17.7	11.8	44.2	35.3	61.8	2.9	61.8	79.5

[a]Rif- Rifampicin (30 µg); Kan- Kanamycin (30 µg); Str- Streptomycin (30 µg); Erythromycin (15 µg); Ttc- Tetracycline (30 µg); Gen- Gentamicin (10 µg); Van- Vancomycin (30 µg); Chc- Chloramphenicol (30 µg); Amp- Ampicillin (10 µg); Neo- Neomycin (5 µg); R- resistance

Table 4: Antimicrobial activity of enterococci against indicator strains

	EA5	EF CCM7420	LMG 13568	LM CCM4699	LM 2024	LM 7223	LM 7562	SA5	S.a. 3A2	S.a. 3A3	S.a. 5A2	*S.e.* ser. *Enteritidis* PT4
EF M1C	-	-	-	-	-	-	-	-	-	+	+	-
EF M2C	+	+	-	+	-	-	-	-	-	+	+	+
EF M7C	-	-	-	+	-	-	-	-	-	-	-	-
EF M7b	-	-	-	-	-	-	-	-	+	-	+	-
EF M4C	-	-	-	+	-	-	-	-	-	-	-	+
EF M6C	+	+	-	-	-	-	-	-	+	-	-	+
EF M5a	+	+	-	-	-	-	-	-	+	+	+	-
EF M3b	+	-	-	+	-	-	-	-	+	-	-	+
EF M1b	-	-	-	-	-	-	-	+	-	-	-	-
EF M2a	-	-	-	+	-	-	-	-	-	-	-	-
EF M2cA	+	-	-	+	-	-	-	-	-	-	-	+
EF M2cB	+	-	-	+	-	-	+	-	-	-	-	-
EF M3a	-	-	-	-	+	+	+	-	+	+	-	-
EF M4aA	-	-	-	-	-	-	-	-	+	+	-	+
E.sp.M4aB	+	-	-	-	-	-	-	+	-	-	-	-
E.sp.M5aA	-	-	-	-	+	+	+	-	-	+	+	-
EF M5aB	-	+	-	-	-	-	-	-	-	-	-	-
E.sp. M6b	+	-	-	-	-	-	-	-	-	-	-	-
E.sp.M7bA	-	-	-	-	-	-	-	-	-	-	-	+
EF M7bB	-	-	-	+	+	+	+	-	-	-	-	-
E.sp.1BM	-	-	-	+	-	-	-	-	-	-	-	-
E.sp.3AM	-	-	-	+	-	-	-	-	-	-	-	-
EF4BM1	-	-	-	+	-	-	-	-	-	-	-	+
E.sp.4BM2	-	-	-	+	-	-	-	-	-	-	-	-
EF5BM1	-	-	-	-	-	-	-	-	-	-	-	-
E.sp.5BM2	-	-	-	-	-	-	-	-	-	-	-	-
EF M1B	-	-	-	-	-	-	-	+	-	+	+	-
E.sp.M2c	-	-	-	-	-	-	+	+	-	+	-	-
EF M1c	-	-	-	-	-	-	+	-	-	-	-	+
E.sp.M5a	-	-	-	+	-	+	+	-	-	+	-	+
EF M6c	-	-	-	+	-	-	-	-	-	+	+	-
E.sp.M3A	-	-	-	-	-	-	-	-	-	+	+	-
EF M4B	-	-	+	+	-	-	-	-	-	-	+	+
EF M2A	-	-	+	-	-	-	-	-	-	+	+	-

EF- *Enterococcus faecium;* EA- *Enterococcus avium;* E.sp.- *Enterococcus species;* + inhibitory zone around indicator organism, - inactivity; *E. avium* EA5 (our isolate; piglet); *E. faecium* CCM 7420 (our isolate; faeces of dog); *S. aureus* SA5, 3A2, 3A3, 5A2 (our isolates, faeces of rabbit); *Listeria innocua* LMG 13568 (Colection of microorganisms of Gent, Belgium); *L. monocytogenes* CCM 4699 (Czech Collection of Microorganisms; Brno); *L. monocytogenes* 2024, 7223,7562 (isolates from food, Veterinary Institute, Olomouc, Czech Republic); *Salmonella enterica* ser. Enteritidis PT4 (Dr. Šišák; Brno, Czech Republic)

faecium (EF) M1C and M2C only *Ent* B gene has been detected and in the strain *Enterococcus* sp. 3AM only *Ent* A gene has been found (**Table 2**); *E. faecium* M7C has possessed *Ent* P, *Ent* B genes. Moreover, in the strain *E. faecium* M3a the genes for *Ent* A and *Ent* B production have been detected. On the other hand, the most

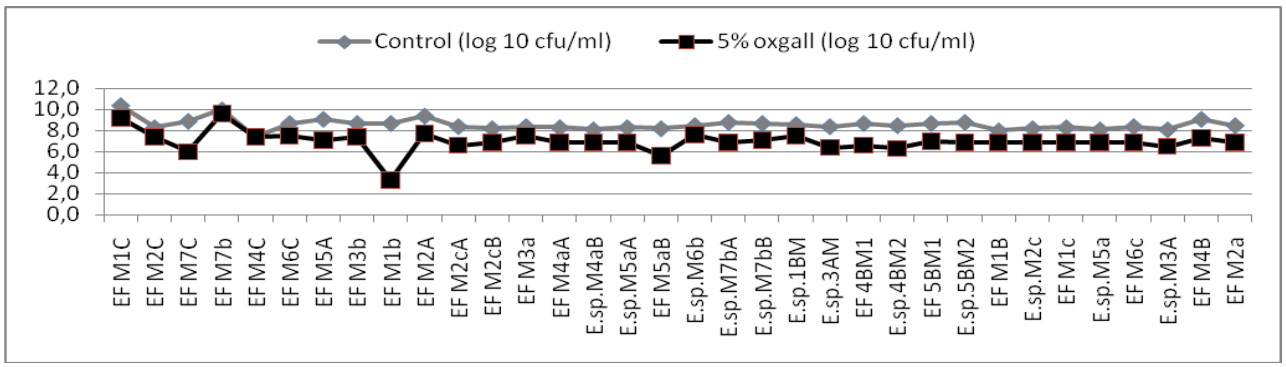

Figure 1: The resistence of bile (5% oxgall) of tested rabbit meat enterococci (EF-*Enterococcus faecium;* E.sp.- *E. species*) expressed in log 10 cfu/ml

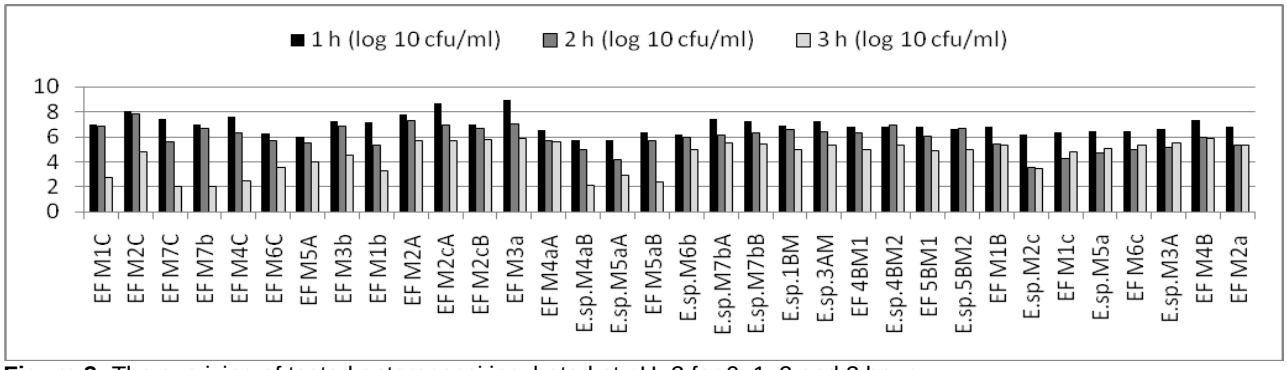

Figure 2: The surviving of tested enterococci incubated at pH=3 for 0, 1, 2 and 3 hour

frequently *Ent* B gene has been found (EF M1C, M2C, M7b, M3a), followed by *Ent* A gene (EF M3a, E. sp. 3AM) and *Ent* P gene (EF M7C).It is interesting, that in spite of the fact that in 85.3 % of It our isolates *Ent* genes have been not found, the growth of at least one indicator strain has been inhibited by our enterococcal isolates (Table 4); however, *E. faecium* EF 5BM1 and *Enterococcu*s sp. 5BM2 did not inhibit the growth of the indicator strains (they did not produce bacteriocin against the indicators used. In summary, the growth of 12 indicators (including *Listeria monocytogenes,* **Table 4**) has been inhibited by bacteriocin produced by our enterococci. Of 34 isolates, 15 strains (44.1 %) have showed the antimicrobial activity against *L. monocytogenes* CCM 4699, 12 strains (35.2 %) against *S. aureus* 3A3, 10 strains (29.4 %) against *S. aureus* 5A2 as well as *Salmonella enterica* serovar Enteritidis PT4. Nine enterococci have inhibited the growth of 2 indicators, 6 strains have showed an inhibitory activity against 3 indicators (**Table 4**).

On the other hand, only 9 strains among 34 (26.5 %) have shown an inhibitory activity against the principal indicator strain *E. avium* EA5. However, 6 indicator strains have been inhibited by the substance produced by *E. faecium* M2C and M3a; those enterococci have possessed *Ent* B gene (the strain EF M2C); respectively *Ent* A, *Ent* B genes (the strain EF M3a). Although, *E. faecium* M5a, *Enterococcus* sp. M5aA and M5a have shown an inhibitory activity against 5 indicators (Table 4), *Ent* genes have been not detected there (Table 2). Enterococci have been able to grow in the presence of 5% oxgall (bile) reaching 10^3-10^9 cfu/ml in comparison with the control growth (10^8-10^{10} cfu/ml)- the strains have grown in oxgall-free broth (**Figure 1**). It means, they have resisted bile (38% to 96%) as well as low pH 3 - the surviving of enterococci in pH 3.0 have ranged from 2.0 up to 5.9 log10 cfu/ml for 3.0 h incubation at 37 °C (**Figure 2**).

Lactid acid production of enterococci has ranged from 0.740 ± 0.091 to 1.720 ± 0.095 mmol/l (**Table 2**). Enterococci have been sensitive to rifampicin (100 %), streptomycin (82.3 %), chloramphenicol (93.1 %), gentamicin (64.7 %), erythromycin (88.2%) and tetracycline (55.8%). On the other hand, besides kanamycin resistance (97.0%), 61.8 % strains have been resistant to ampicillin, 79.5 % to neomycin; 61.8 % to vancomycin (**Table 3**).

DISCUSSION

Among enterococci, *E. faecium* seems to be the dominant cultivable enterococcal species in rabbits. Prevalence of *E. faecium* in faeces of rabbits (50 % of 58 isolates) has been also described by Simonová *et al.* (2005). In general, *E. faecium* and *E. faecalis* represent the most frequently occurred species among enterococci from

different sources (Franz *et al*., 1999, 2003). Strompfová and Lauková (2007) reported occurrence of these species also in poultry. The species mentioned have also formed predominant colonization of enterococci in piglets; among 55 enterococcal isolates, 67.3% strains were *E*. *faecium* (Strompfová and Lauková, 2009) and they have been also reported in healthy dogs (Strompfová *et al.*, 2006). Concerning their occurrence in food matrix, in general, the amount of enterococci in rabbit meat is in accordance with that count reported for meat products by Lauková *et al.* (2005). Franz *et al.* (2003) has referred enterococci as probiotic bacteria in different food. The important factor for the evaluation of enterococci as further probiotics is their antimicrobial (bacteriocin) activity against spoilage bacteria. For example, the ability of rabbits enterococci to produce bacteriocin-like substance active against the target of Gram-positive as well as Gram-negative strains was described by Simonová and Lauková (2007).

In addition, strong antilisterial activity of bacteriocins produced by enterococci has also been reported previously in several matrices *e.g.* cheese, fermented meat products, canine feed, horses and rabbits faeces (Franz *et al.*, 1999; Lauková *et al.*, 2003, 2008a, 2008b; Mareková *et al.*, 2003; Simonová *et al.*, 2005). In our study, some of strains, *Enterococcus* sp. M5a, E. sp. M5aA*, E. faecium* M5a have shown an inhibitory activity against 5 of 12 indicator strains; however, they have not possessed enterocin genes. It seems, we can discussed silent genes (Strompfová *et al.*, 2008) or the strains can produce the different bacteriocins as were tested or they inhibited the growth of the indicators due to other antimicrobial agents than bacteriocins (*e.g.* lactic acid). In this study, *Ent* L50B gene was not detected in enterococci; oppositelly, in faecal enterococci (from rabbits), *Ent* L50B was the most frequently detected followed by *Ent* A gene (Strompfová *et al*., 2008). Therefore, it is possible that some strains with bacteriocin activity could probably produce new substance.

Commonly, probiotic potential of strains is tested at least by their resistance to bile or low pH. Hyronimus *et al.* (2000) tested bile tolerance and resistance to acids among spore-forming LAB (SFLAB). He concluded that some SFLAB survive in acid conditions (pH 2.5-3.0) and few SFLAB are weakly tolerant to 0.3 % bile. Our enterococci were found with survival rate at pH 3.0 after 3 h (in the range 10^3-10^9 cfu/ml). Similar results were achieved in enterococci isolated from the gastrointestinal tract of chickens (Strompfová and Lauková 2007) as well as from rabbits' faeces (Simonová, 2006). Rincé *et al*. (2003) presents results from analysis of the physiological response of *E. faecalis* ATCC 1943 towards bile salts (susceptibility, homologous tolerance and cross-protections against heterologous stresses) and the identification of genes involved this response. Analysis of *E. faecalis* susceptibility towards the bile salts has given evidence for an extremely rapid killing effect. Compared to kinetics observed for action of lethal challenges-heterologous stresses by heat, ethanol, NaCl, H_2O_2, sodium dodecyl sulfate (SDS), acid pH and alkaline pH, such as a rapid killing effect was only observed with SDS and suggested that the bile salts probably act by solubilization of membrane components.

LA production by enterococci from rabbit meat was similar to the other enterococci, *e*.g. from canine feed (Lauková *et al.*, 2008a), horses (Lauková *et al.*, 2008b) and rabbit faeces (Simonová, 2006). Bacteriocin-producing strains with probiotic potential are requested to be absent of antibiotic resistance genes (Klein, 2011). Our strains showed kanamycin (aminoglycoside) resistance but this intrinsic resistance is in enterococci obligatory (usually chromosomally encoded) (Butaye *et al.*, 2001; Kak and Chow, 2002; Klare *et al.*, 2002). Majority of strains were antibiotic sensitive. Lower range of vancomycin resistant enterococci (VRE; 61.8 %) is curious, but the presence for *Van* genes was not tested and strains which were selected for further studies are vancomycin sensitive. Acquired type resistance of enterococci to vancomycin has been presented by Leclerq (1997).

VRE in humans are a problem not only because of health, but also because of a possible transmission of VRE from the matrix of domestic animals to humans and/or through the food chain. Acquired type of resistance to vancomycin is conditional on the presence of genes *Van* A, *Van* B, *Van* D, *Van* E, *Van* G; *Van* A gene and *Van* B are found primarily in bacterial species *E. faecium* and *E. faecali* (Morrison *et al.*, 1997, Méndez-Alvarez *et al.*, 2000). Among enterococcci from piglets, similarly as in our study, more strains were sensitive to tetracycline, chloramphenicol, erythromycin, rifampicin. Moreover, they were also vancomycin sensitive (Strompfová and Lauková, 2009). Simonová (2006) reported resistance of enterococci from rabbit faeces to kanamycin and vankomycin.

CONCLUSION

Of 34 isolates (mostly allotted to the species *E. faecium)*, 15 strains (44.1 %) have shown the antimicrobial activity against *L. monocytogenes* CCM 4699, 12 strains (35.2 %) against *S. aureus* 3A3, 10 strains (29.4 %) against *S. aureus* 5A2 as well as *Salmonella enterica* serovar Enteritidis PT4. The structural genes for *Ent* A, B, P production have been detected in the isolated enterococci. The strain *E. faecium* M3a has produced bacteriocin with an inhibitory activity against *L. monocytogenes* and *S. aureus*. From the basic point of view, it has been selected for its more detail characterization with the aim for its application possibility *e.g*. as feed additive. Of course, the following experiments have been requested.

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