ENTEROCOCCI FROM PANNON WHITE RABBITS: DETECTION, IDENTIFICATION, BIOFILM AND SCREENING FOR VIRULENCE FACTORS

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Abstract: Properties of enterococci isolated from the Hungarian breed Pannon White were studied to spread knowledge regarding the properties of microbiota in rabbits from the basic research standpoint and to select a beneficial candidate for application in husbandry. Faeces from 113 Pannon White rabbits (mostly maternal line and some paternal line) were collected. They were sampled from rabbits varying in age and sex (82 kits, 6 does, 6 bucks, 19 adult rabbits), which were aged 2 wk (14 suckling rabbits), 6 and 8 wk (68 weaning and post-weaning rabbits), adult rabbits (31, one year). Faecal mixtures were sampled into sterile packs with faeces from 5-6 animals in each. The total count of enterococci from these Pannon White rabbits reached, on av. 5.28±0.29 colony forming units/g (log10). Among the 19 enterococci, 14 *E. faecalis* and 5 *E. faecium* were detected using 3 identification methods. The enterococci were mostly resistant to antibiotics. They were non-haemolytic, Dnase and urease negative. They did not form biofilm. They were free of the h*ylEfm* gene and *IS16* genes; the most frequently detected genes were *gelE, efaAfm, efaAfs*. Based on these results, *E. faecium* EF9a was selected for further analysis.

Key Words: Pannon White, identification, enterococci, properties, rabbits.

INTRODUCTION

Broiler rabbits are farmed for their nutritionally acceptable meat (Chrastinová *et al.,* 2010). Rabbit is considered one of the healthiest meats because of its easy digestion, high protein values (20-21%), unsaturated fatty acids, minerals such as potassium, phosphorus and magnesium and its low fat, cholesterol and sodium contents (Dalle Zotte, 2002; Szabóová *et al.,* 2012). The rabbit intestine is colonised by abundant microbiota, predominantly strictly anaerobic bacteria (Marounek *et al.,* 2000; Bagóne Vantus *et al.,* 2018). However, the composition of the rabbit gastrointestinal tract (GIT) environment changes with age and also in association with the weaning period. However, most of the GIT microbiota is formed by lactic acid-producing bacteria from the phylum Firmicutes, to which representatives of the genus *Enterococcus* also belong (Simonová and Lauková, 2004, 2007). Regarding the enterococcal species and their properties originating from breeds reared in Slovakia, several studies have been already published (Simonová and Lauková, 2004, 2007; Simonová *et al.,* 2005; Lauková *et al.,* 2012) reporting their beneficial properties such as probiotic character or bacteriocin production; however, the occurrence of virulence factor genes or production of biofilm have also been evaluated (Pogány Simonová and Lauková, 2017; Bino *et al.,* 2018).

Development in animal breeding induces the search for new technologies to maintain healthy rabbits and improve welfare. Moreover, broiler rabbits are a useful animal model for their ease of handling. Based on our *in vitro* results,

therefore, bacteriocin-producing *Enterococcus faecium* strains with probiotic properties (isolates from our laboratory, from previous studies) were applied in broiler rabbits with beneficial effects on the immune system and reduction of undesirable bacteria. After their application, improvement in growth performance was noted without negative impact on carcass quality (Laukavá *et al.,* 2012; Pogány Simonavá *et al.,* 2013).

As rabbit breeding has a long tradition in Hungary (Szendro and Bleyer, 1999) and focusing on our co-operation with Hungarian colleagues, we decided to check the properties of enterococci isolated from the Hungarian breed Pannon White to extend the knowledge on the microbiota in rabbits from the basic research point of view.

MATERIALS AND METHODS

Sampling and strains identification

Faeces from 113 Pannon White rabbits (mostly maternal line and some paternal line) were collected. They were sampled from rabbits of various ages and sex (82 kits, 6 does, 6 bucks, 19 adult rabbits), which were aged 2 wk (14 suckling rabbits), 6 and 8 wk (68 weaning and post-weaning rabbits), adult rabbits (31, one year). Faecal mixtures were sampled in sterile packs with faeces from 5-6 animals in each. The rabbits were reared under standard conditions in mesh-wired cages with free access to water and feed *(ad libitum)*. They were fed with commercial feed from Cargill (USA). The temperature was 16-18°C and the photoperiod was set to 16 h of light and 8 h of dark. Sampling of faeces was carried out during August and October 2009. Handling of the animals and sampling followed the rules set by the Slovak Veterinary and Food Administration and approved by the Ethics Commission of Káposvár University and the appropriate Hungarian Administration. Faecal samples were treated using the standard microbial dilution method according to the International Organization for Standardization (ISO). Briefly, faeces (10 g of each sample) were decomposed in 90 mL of Ringer solution (ratio 1:9, Oxoid, the United Kingdom), diluted and plated onto M-*Enterococcus* agar (Difco, USA). The representative colonies were picked up, checked for purity and stored for further analyses using the Microbank system (Pro-Lab Diagnostic, Richmond, Canada).

Grown colonies on M-*Enterococcus* agar were picked up and monitored for their purity by plating on Brain-heart agar (BHA, Difco, USA) supplemented with 5% of defibrinated sheep blood. Species identification was performed using the MALDI Biotyper™ identification system (Bruker Daltonics, USA) based on analysis of bacterial proteins using matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Lysates of bacterial cells were prepared according to the producers' instructions (Bruker Daltonic 2011) prior to identification. The results were evaluated using the identification database considering scores between 2300-3000 as indicating high secure species identification, then between 2000-2299 as indicating secure probable genus identification/probable species identification and scores between 1700-1999 as indicating probable genus identification. Positive control strains were those included in the identification database.

Moreover, genotyping was performed using the primer pairs according to Woodford *et al.* (1997) and polymerase chain reaction (PCR; Thermocycler Techne, the United Kingdom). The sequences of the primer pairs used for DNA amplification of *Enterococcus faecalis* were 5'-ATCAAGTACAGTTAGTCTT-3' and 5'-ACGGATTCAAAGCTAACTG-3' (Invitrogen). For *E. faecium,* the sequences of the primer pairs were as follows: 5'-GCAAGCTTCTTAGAGA-3' and 5'-CATCGTGTAAGCTAACTTC-3' (Invitrogen). Initial denaturation was carried out at 95°C for 2 min, 40 cycles of 95°C for 1 min, 55 °C for 1 min, 72°C for 1 min, 72°C for 10 min. *E. faecium* CCM7419 (our strain deposited with the Czech Culture Collection of Microorganisms (CCM)) and *E. faecalis* CCM4224 (CCM) were used as positive controls.

In addition, phenotype characterisation was performed using the commercial BBL Gram-positive Crystal kit (Becton and Dickinson, Cockeysville, USA) to confirm the species following the properties reported in De Vos *et al.* (2009).

DNase activity, haemolysis and urease activity

To determine DNase (nuclease activity), the surface of DNase agar (Oxoid, USA) was inoculated with each strain and incubated for 24 h at 37°C. Colonies producing DNase hydrolysed the deoxyribonucleic acid (DNA) contained in the medium. After medium flooding and acidifying with 1 N HCl, the DNA precipitated and the medium became turbid,

then cleared zones formed around DNase-positive colonies. *S. aureus* SA4 from dog (our strain-Dr. Strompfová) was used as positive control.

Haemolysis was detected by streaking the cultures on BHAgar (Difco, USA) supplemented with 5% defibrinated sheep blood. Plates were incubated at 37°C for 24-48 h under semi-anaerobic condition. Presence or absence of clearing zones around the colonies was interpreted as α, β-haemolysis and no clearing zone was evaluated as negative γ-haemolysis, respectively (Semedo-Lemsaddek *et al.,* 2003).

Urease [EC 3.5.1.5.] activity is a metabolic property which was analysed using the quantitative spectrophotometric method (A_{640}). This method is based on reaction mixture dying (cultivated broth culture with 2M urea solution) with phenol-hypochlorite reagents. It is expressed in nkat/mL; 1 katal corresponds to the enzyme amount which converts 1 mole of substrate in 1 s. Results were calculated from 3 independent measurements.

Antibiotic resistance/susceptibility phenotype

The identified enterococci were tested for their antibiotic phenotype profile using the agar disk diffusion method and antibiotics as recommended for enterococci in the Clinical Laboratory Standard Institute guidelines (CLSI, 2012). BHA (Oxoid) supplemented with 5% sheep blood was used and the following antibiotic disks with concentrations recommended by the supplier: clindamycin (DA-2 µg), ampicillin, gentamicin, (AMP, CN, 10 µg), penicillin-P (10IU), azithromycin, erythromycin (AZ, E, 15 µg), tetracycline, vancomycin, chloramphenicol, rifampicin (TC, VA, C, RA, 30 µg). The antibiotic disks were supplied by Oxoid and Becton and Dickinson (United Kingdom and USA). The inhibition zone diameter for individual antibiotics was assessed as susceptible or resistant according to CLSI (2012) and the interpretation table of the antibiotic disk producers. The inhibition zones diameters were evaluated and compared with the reference type strain included in the supplier documentation.

Virulence factor genes detection

First, the gelatinase phenotype test was performed as follows: Todd-Hewitt agar with 3% gelatin (Becton and Dickinson, Cockeysville, USA) was used for growing the tested strains. After their growth (48 h at 37°C), the agar plates were flooded with a 15% solution of $HgCl_2$ (in 20% HCl). Loss of turbidity halos around colonies was checked at 4°C (Kanemitsu *et al.,* 2001).

Genes for seven virulence factors were screened using polymerase chain reaction (PCR) amplification with the primers and conditions reported by Ribeiro *et al.* (2011) and Kubašová *et al.* (2017). Genes for the following virulence factors were checked: *gelE* (gelatinase), *esp* (enterococcal surface protein), *efaAfm* (adhesin *E. faecium*), *efaAfs* (adhesin *E. faealis), cylA (*cytolysin A*), HylEfm* (hyaluronidase*), IS16* (element IS). The fragments of PCR products were visualised by UV light. The positive controls were the strains *E. faecalis* 9Tr1 (our strain, Lauková *et al.,* 2015), *E. faecium* P36 (kindly supplied by Dr. Semedo-Lemsadek, University Lisbon, Portugal and by Dr. Klare from Robert Koch University, Germany).

Biofilm formation on Congo red agar

To test biofilm formation in our identified enterococci, the qualitative method through growth of strains on Congo red agar was used according to Freeman *et al.* (1989). The cultivation medium was composed of Brain-heart infusion (Difco, Michigan, USA, 37 g/L) enriched with sucrose (36 g/L), pure agar (30 g/L) and Congo red dye (0.8 g/L, Merck, Germany). The medium was autoclaved at 121°C for 15 min. Plates of the medium were inoculated with the tested enterococcal strains and incubated at 37°C for 24 h. A positive result was indicated by black colonies with a dry crystalline consistency. Non-slime producers usually remained pink. The colour was also checked after 48 and 72 h.

RESULTS

The total count of enterococci from Pannon White rabbits reached 5.28±0.29 colony forming units (cfu)/g (log10), on av. The average count of enterococci detected in faecal samples of baby rabbits (kits) reached 5.75±0.39 cfu/g (log10), meaning animals aged 2, 6 and 8 wk. In dam rabbits, sire rabbits and rabbits aged one year the enterococci reached

a count of 4.82±0.19 cfu/g on av. This indicates that enterococci were well balanced in rabbits regardless of their age.

Based on their MALDI-TOF MS identification scores, identical strains were eliminated from further testing. Finally, 19 enterococcal strains were studied. The prevalence of *E. faecalis* species (14 strains) was identified. Five strains were allotted to the species *E. faecium*. Score values for the identified *E. faecalis* strains ranged from 2300 up to 2456; only *E. faecalis* EE15a was detected with a score of 1.801 (Table 1). The strains allotted to the species *E. faecium* were evaluated with scores ranging from 2.095 up to 2.271 (Table 1). The species assignment was also confirmed using PCR analysis; the 941 bp band was visualised for *E. faecalis* and the 550 bp band for *E. faecium*. In *E. faecalis* strains the phenotypic properties checked were in association with the metabolic properties of the reference strain *E. faecalis* ATCC19433 (e.g. positive reaction to 4MU-β-D-glucoside, L-tryptophan-AMC, esculin, arginine, fermentation of trehalose, lactose, sucrose, mannitol, fructose, glycerol, sorbitol, arabinose; negative reaction in L-valine-AMC, L-arginine-AMC, 4MU-β-D glucuronide, L-isoleucine-AMC and variable for urea), while for *E. faecium* (*E. faecium* CCM7419) sorbitol and raffinose were originally negative.

The strains showed negative DNase activity and haemolysis, except *E. faecium* EF21a which showed β-haemolysis (Table 1). Ten strains were urease negative/not ureolytic and 9 strains possessed low urease activity, less than, on av. 1.0 nkat/mL; 0.47±0.08 nkat/mL (Table 1).

Enterococci were susceptible to vancomycin (inhibition zone size diameters ranged from 11 to 15 mm), penicillin (15-22 mm) and chloramphenicol (15-28 mm). On the other hand, they were tetracycline and kanamycin resistant. Most strains were also resistant to clindamycin, gentamicin, azithromycin and erythromycin; however, they were mostly susceptible to rifampicin and ampicillin. *E. faecium* EF9a displayed the highest susceptibility to antibiotics (Atbs). This strain was susceptible to 8 Atbs from 11 used/resistant only to 2 from 11 used Atbs respectively (kanamycin and tetracycline, Table 2). In contrast, the most resistant was *E. faecalis* EE13a strain, which was resistant to 8 from 11 Atbs. Eleven strains were resistant to 6 Atbs, 3 strains showed resistance against 5 Atbs and 2 strains were resistant to 4 Atbs. In spite of susceptibility to chloramphenicol, penicillin and vancomycin, the isolated enterococci were mostly resistant to Atbs.

Table 1: Identification of enterococci from Pannon White rabbits and their properties.

Strains	Score	Haemolysis	Biofilm	Urease
EE5/a	2.300	neg	neg	0.64±0.08
EE6/b	2.434	neg	+	0.32±0.05
EE8/b	2.322	neg	neg	0
EF9a	2.442	neg	neg	0
EE9c	2.440	neg	neg	0
EE10a	2.420	neg	neg	0
EE10c	2.456	neg	neg	0.32±0.05
EE11a	2.309	neg	neg	0
EE11b	2.400	neg	neg	0.32±0.05
EE13a	2.305	neg	neg	0
EE14a	2.351	neg	neg	0.64±0.08
EE15a	1.801	neg	neg	0
EF16a	2.095	neg	neg	0
EE17a	2.300	neg	+	0
EF17b	2.310	neg	neg	0.32±0.05
EE19b	2.421	neg	neg	0
EE20b	2.426	neg	neg	0.68±0.09
EF21a	2.271	β	neg	0.68±0.09
EF22b	2.261	neg	neg	0.32±0.05

EE-*Enterococcus faecalis*, EF-*E. faecium*; neg-negative; β- haemolysis; + biofilm formation on Congo red agar (growth), urease in nkat/mL±standard deviation.

Table 2: Antibiotic phenotype profile of enterococci from Pannon White rabbits.

Strains	DA	AMP	GN	AZM	E	RIF
EE5/a	R	S	R	R	R	S
EE6/b	R	S	R	S	S	S
EE8/b	R	S	R	S	R	S
EF9a	S	S	S	S	S	S
EE9c	R	S	R	R	R	S
EE10a	R	S	R	R	R	S
EE10c	R	S	R	R	R	S
EE11a	R	S	R	R	R	S
EE11b	R	S	R	R	R	S
EE13a	R	R	R	R	R	R
EE14a	R	S	R	R	R	S
EE15a	R	S	R	R	R	S
EF16a	R	S	R	R	S	S
EE17a	R	S	R	R	R	S
EF17b	R	S	R	R	S	S
EE19b	R	S	R	R	R	S
EE20b	R	S	R	S	S	S
EF21a	R	S	R	R	R	S
EF22b	R	S	R	R	R	S

EE-*Enterococcus faecalis,* EF-*E. faecium*; Enterococci were resistant to tetracycline and kanamycin. They were susceptible to vancomycin, penicillin, chloramphenicol. DA-clindamycin (2µg), AMP-ampicillin, GN-gentamicin (10µg), AZM-azithromycin, E-erythromycin (15µg), rifampicin (30 µg), R-resistant, S-susceptible.

Phenotype gelatinase testing showed 11 strains to be gelatinase positive among the 19 tested (Table 3). *GelE* gene was detected in 8 of those 11 strains with positive gelatinase phenotype. In 3 strains (EE10a, EE10c, EE20b) which had negative gelatinase phenotype, *gelE* gene was detected (Table 3). The opposite situation was noted in the strains EE14a, EF17b and EF22b, where *gelE* gene was not detected, although these strains were gelatinase positive using phenotype testing. In 13 strains the presence of *efaAfm* and *efaAfs* genes was noted. Six strains possessed the *cylA* gene, and the *esp* gene was detected in 2 strains (EE20b and EE22b). The enterococci were free of h*ylEfm* gene and *IS16* gene. *E. faecalis* EE8b, EE5/a and *E. faecium* EF9a possessed 1 gene in each strain (Table 3). Four genes from the 7 tested were harboured by 8 strains from the 19 tested. However, the strains were biofilm non-forming, except 2 *E. faecalis* strains EE6/b and EE17a, both biofilm forming using the Congo red agar method (Table 1).

DISCUSSION

Although enterococci belong in the obligatory microbiota in the GIT of animals, their counts can differ. The total counts of enterococci isolated from Pannon White rabbits were at the same level as those found in farm rabbits in Slovakia (Simonová *et al.,* 2005, Lauková *et al.,* 2018). Moreover, the faecal enterococcal counts detected from Pannon White rabbits are similar to those also enumerated in other animals such as pheasants, ostriches, dogs and horses (Kandričáková *et al.,* 2015; Lauková *et al.,* 2008, 2016; Kubašová *et al.,* 2017) or in ruminants (Lauková *et al.,* 1990). However, it is interesting that Simonová *et al.* (2005) evaluated 50% of strains as *E. faecium*, followed by 19% *E. faecalis* strains; while in our study involving Pannon White rabbits the representatives of the species *E. faecalis* dominated. Linaje *et al.* (2004) also reported the species *E. faecalis* and *E. faecium* as the most frequent enterococcal species occurring in the rabbit intestinal system. Despite the fact that nowadays more than 55 enterococcal species have been validated, (including two new species in 2017), the species *E. faecalis* and *E. faecium* are predominant inhabitants in the GIT of animals, independently of the animal species.

Strain identification using the MALDI-TOF MS system evaluated 15 strains with scores between 2300-3000, corresponding to high secure species identification, 3 strains were evaluated with scores between 2000-2299, corresponding to secure probable genus identification/probable species identification. Only strain EE15a was

Table 3: Gelatinase phenotype and virulence factor gene determination in faecal enterococci from Pannon White rabbits.

Strains	gel/ph.	*gelE*	*efaAfm*	*efaAfs*	*cylA*	*esp*
EE5/a	-	-	+	-	-	-
EE6/b	+	+	+	+	-	-
EE8/b	-	-	+	-	-	-
EF9a	-	-	-	-	+	-
EE9c	-	-	+	+	+	-
EE10a	-	+	+	+	+	-
EE10c	-	+	-	+	+	-
EE11a	+	+	-	+	-	-
EE11b	+	+	+	+	-	-
EE13a	+	+	-	+	+	-
EE14a	+	-	+	-	-	-
EE15a	+	+	+	+	-	-
EF16a	+	+	+	+	-	-
EE17a	+	+	+	+	-	-
EF17b	+	-	+	-	-	-
EE19b	+	+	-	+	-	-
EE20b	-	+	-	+	-	+
EF21a	-	-	+	+	+	+
EF22b	+	-	+	-	-	-

EE-*Enterococcus faecalis*, EF-*E. faecium*; *hylEfm*-all strains were negative/free of this gene; IS16-all strains were free of this gene; gel/ph.-gelatinase phenotype; gelE-gelatinase, efaAfm-adhesin *E. faecium*, efaAfs-adhesin *E. faecalis*, cylA-cytolysin A, esp-surface protein; +:gene found; -:no gene; gel/ph:gelatinase phenotype.

evaluated with a score in the range 1700-1999, which is in accord with probable genus identification. MALDI-TOF MS is an emerging next-generation high-quality technology applied in bacterial strain identification which is successfully used in screening labs as well as in research (Neelja Singhal *et al.* 2015). However, the spectral database containing peptide mass fingerprints of the type strains of specific genera/species/subspecies/strains needs to be upgraded to include newly-validated species. For this reason, other analyses are usually added to confirm precise identification. In our study, PCR analysis using the appropriate primers confirmed the species identification and their taxonomic assignment. Moreover, the phenotype testing performed, associated with the characteristics set out in Bergey's Manual of Systematic Bacteriology (De Vos *et al.,* 2009), confirmed our genotyping as well as the MALDI-TOF identification.

The identified strains were DNase negative; β-haemolysis was detected in the EE17a strain. In general, if enterococci are haemolytic, then α-haemolysis is more typical for *E. faecium*. Moreover, in our previous study testing faecal enterococci from ostriches the strains were mostly non-haemolytic, and only 2 *E. hirae* strains formed β-haemolysis (Lauková *et al.,* 2016). Urease is an enzyme produced by some bacteria hydrolysing urea from material into ammonia in order to regulate the pH, e.g. in the bacterial biofilm. Urease activity is a typical property of ruminal enterococci, for example. In ruminal enterococci from mouflons or European bison, enterococcal urease activity ranged from 2.47±0.06 to 5.59±0.04 nkat/mL, while in enterococci from the rumen content of deer it was higher, ranging from 3.57±0.8 to 31.80±2.1 nkat/mL (Lauková, 1995; 1999). Faecal strains can be urease active as indicated in our strains; however, rare and low urease activity has been also measured.

Eleven strains possessed the *gelE* gene. Gelatinase or extracellular metalloprotease is able to hydrolyse gelatin, collagen and haemoglobin, which has also been attributed to bacterial adherence and biofilm formation (Kayaoglu and Orstavik, 2004). However, our gelE positive strains did not form biofilm, except for the EE6/b and EE17a strains. The adhesin *E. faecium* (*efaAfm*) gene was detected in all *E. faecium* strains except the EF9a strain, but it was also present in *E. faecalis* strains. A similar situation was described by Lauková *et al.* (2015) in faecal *E. faecium* and *E. thailandicus* strains isolated from beavers. On the other hand, the adhesin *E. faecalis* (*efaAfs)* gene was present

in 2 *E. aecium* strains from the 5 identified. The *Esp* adhesin gene was detected in 1 *E. faecium* strain (EF21a) and 1 *E. faecalis* strain (EE20b). This adhesin contributes to enterococcal biofilm formation, which could lead to resistance to stress in general. Our strains, as mentioned above, did not form biofilm, not even the EE20b and EF21a strains in which the *esp* gene was detected. Latasa *et al.* (2006) reported that esp-negative *E. faecalis* strains were able to produce biofilm after receiving plasmid transfer of the *esp* gene. The esp adhesin is thought to promote adhesion and evasion of the immune system and to play a role in antibiotic resistance (Foulquie-Moreno *et al.,* 2006). Our 2 *esp* positive strains were resistant to 6 Atbs. Kubašová *et al.* (2017) found the most frequently occurring gene-encoding adhesin to be *efaAfm* (95.7%) in faecal canine-derived *E. faecium* strains and *efaAfs* (91.4%) in *E. faecalis* strains. The cytolysin production determinant *cylA* was found in 6 strains including the representatives of both species. That is β-haemolysin, which is capable of lysing many prokaryotic cells, erythrocytes and other eukaryotic cells (van Tyne and Gilmore, 2014). Hyaluronidase has been associated with virulence of enterococci in host tissue invasion; it acts on hyaluronic acid and is a degradative enzyme which is associated with tissue damage. IS16 is a specific marker for hospital-associated enterococci (Kyaouglu and Orstavik, 2004). Our strains were free of gene-encoding hyaluronidase (*hylEfm*) as well as element *IS16* gene. Rare occurrence of these genes in animal/canine-derived strains was also reported by Kubašová *et al.* (2017).

Resistance to KAN is chromosomally encoded in enterococci, so it was not surprising that our strains were resistant to KAN. However, our strains displayed phenotypical resistance to TC similar to the prevalence of TC resistance found in *Enterococcus* isolates from food (Huys *et al.,* 2004). Rice *et al.* (1991) described the existence of GN resistance genes in *E. faecalis* human strains which were integrated into the bacterial chromosome. This may explain why 18 of our 19 strains showed phenotypical resistance to GN. Our strains were susceptible to VAN, C and P. Similarly, no genes conferring resistance to VAN were reported by Splichalova *et al.* (2015) in *E. faecalis* strains from coraciiform birds. Our strains were susceptible to 5 Atbs and resistant to 6 Atbs out of the 11 tested. Furthermore, the gene for Atb resistance (especially genes for VAN resistance) also has to be screened (or the plasmids probably coding it) to exclude transfer possibilities. Based on these results, *E. faecium* EF9a was selected for further analysis to check its potential use in rabbit husbandry.

CONCLUSION

The total count of enterococci from Pannon White rabbits reached, on av 5.28±0.29 cfu/g (log10). Among the 19 enterococci, 14 *E. faecalis* and 5 *E. faecium* were detected using 3 identification methods. These enterococci were mostly resistant to antibiotics; they were DNase, haemolysis and urease negative. They did not form biofilm. They were free of h*ylEfm* and *IS16* genes; the most frequently detected genes were *gelE, efaAfm, efaAfs*. Based on these results, *E. faecium* EF9a was selected for further analysis.

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