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Bacteriocin Production Of *Enterococcus* Species Isolated From Milk And Some Cheese Samples

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Abstract

Enterococci can be used as a starter or probiotic culture in the food industry. However, enterococci are also implicated in severe multi-resistant nosocomial infections. In this study, some probiotic characteristics such as antimicrobial activity and antibiotic susceptibility were investigated.Twenty-three strains of *Enterococcus* species isolated from Raw milk (7 strains), Karish cheese (8 strains), Domiati cheese (5 strains), and Ras cheese (3 strains) were studied for the capability to produce bacteriocin and antibiotic susceptibility.The seven studied antibiotics showed a different powerful effect on *Enterococcus* strains. The most effective antibiotic against 23 *Enterococcus* strains were amikacin, cefotaxime, and cefoxitin, which inhibited the growth of all tested strains, followed by levofloxacin azlocillin, and cloxacillin which inhibited 21, 19, and 19 strains, respectively, out of 23 studied strains. In addition, colistin showed the lowest powerful effect against all *Enterococcus* strains studied was resistant to this antibiotic. Antimicrobial activity was confirmed for all 23 strains against *B. thuringiensis* and 16, 13, 9, 9, 7, 6 out of 23 strains against *Str. pyogenes*, *Ps. aeruginosa*, *B. cereus*, *Staph. aureus*, *E. coli* O157:H7, and *S. typhimurium*, respectively. Strains *E. faecium* Rm5-1, *E. faecium*Rm6-1, *E. durans* Rm3-1, *E. durans* Rm3-2, *E. durans* Kc1-1, *E. durans* Kc5-2, *E. faecalis* Kc2-1, *E. faecium* Dc1-1, *E. faecium*Dc10-2, and *E. durans* Rc3-2 showed potent antimicrobial activity against most indicator strains.

Keywords: *Enterococcus*;*E. faecium*;Bacteriocin production;Antibiotic susceptibility.

1- Introduction

One of the essential types of bacteria which widely grow in raw milk and dairy products isthe *Enterococcus* species. Enterococcus faecium, Enterococcus durans, and Enterococcus faecalis are the most common Enterococcus species found in dairy products [1,2]. Among the several biotechnological properties of Enterococcus species are bacteriocin production, probiotic characteristics, and usability in dairy technology.

Some *Enterococcus* strains play an important role in cheese making and contribute to sensory characteristics. However, in contrast, to lactic acid bacteria, enterococci are not considered GRAS (generally regarded as safe);for example,their presence in water is a sign of fecal contaminationand their resistance to many antibiotics. On the other hand, probiotic propertieswith microbial balance in the gastrointestinal flora in humans and animals and the ability to synthesis proteins with antimicrobial activity such as bacteriocin production represent important functions ofenterococci in fermented food products [3,4]. Therefore enterococci are used in some dairy products, pharmaceutical products used in the clinical treatment of humans, and preparations to avoid enteric diseases in animals [6, 7].In addition, the use of *Enterococcus* strains with probiotic properties as food additives have been proposed in the international meeting of the lactic acid bacteria industrial platform [5].

Ofthe species of enterococci found in dairy products, *Enterococcus faecalis* and *Enterococcus faecium*are the main ones. Theycan produce a variety of enterocins that haveactivity against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium spp*. [8,9,10].

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One ofthereasons for the risk of nosocomial infections related to enterococci might be their ability to develop resistance against a wide variety of antibiotics, including glycopeptide antibiotics (vancomycin and teicoplanin) [11].They also stated that antibiotic resistance combined with the virulence factors (aggregation substance production, gelatinase, hemolysin, and surface proteins) determine that enterococci are not generally recognized as safe (GRAS) microorganisms.

The above information provides the rationale behind this research that aimed to study the resistance of *Enterococcus* species toward antibiotics, the bacteriocin production by *Enterococcus* species, and investigate the probiotic potential of *E. faecium*, *E. durans,* and *E. faecalis* strains isolated from milk and some cheese.

2. Experimental

Materials

Preparation of milk samples

A portion of 1ml of each market milk sample was added to sterilized tubes with 9ml of sterilized physiology solution, and the serial dilution technique was applied.

Preparation of cheese samples

A portion of 1g of each cheese sample was added to the sterilized mortar with 9ml of sodium citrate, then emulsified in it, and the serial dilution technique was applied.

Enterococcusspecies

Studies *Enterococcus* species (23 strains) isolated from Raw milk (7 strains), Karish cheese (8 strains), Domiati cheese (5 strains), and Ras cheese (3 strains) were obtained from the Dairy department faculty of Agriculture, Al-Azhar University. Strains with previously were identified by several of phenotypic: Gram stain, catalase production, growth at 10°C and 45°C and in 6.5% NaCl, pH 9.6 in combination with resistance to bile 40%, Sodium azide 0.04%, 60°C/30min, carbohydrate fermentation and the API 20 Strep test kits (BioMerieux SA, Marcy I' Etoile, France).

Methods

Sensitivity to antibiotics

A total of 23*Enterococcus* species were tested for sensitivity to Amikacin (30 µg), Azlocillin (75 µg), Cefotaxime (30 µg), Cefoxitin (30 µg), Cloxacillin (1 µg), Colistin (10 µg) and Levofloxacin (5 µg). Overnight cultures of tested isolates were streaked onto Tryptic Soy Agar medium (Oxoid). Antibiotic disks were placed on the surface of the agar medium with sterilized forceps and gently touched with the tip of forceps to assure proper contact. The plates were incubated for 16-24 hours at 37(C) and then examined for inhibition zones. Inhibition zone diameters were measured and recorded [12].

Bacteriocin production assay

Cell-free neutralized supernatant was used to test bacteriocin production and was prepared by centrifugation of 18 h culture using Tryptic Soy Broth (Oxoid, UK) of tested strains (10,000 × g for 30 min). Bacteriocin activity was tested by agar spot test using Brain Heart Infusion Agar (Biolife, Italy) against indicator strains *B. cereus, B. thuringiensis, Staph. aureus, Str. pyogenes, E. coli O157:H7, S. typhimurium, Y. enterocolitica* and *Ps. aeruginosa*. The pH of the bacteria suspensions was neutralized. Incubation of the plateswas performed for 24 hrat 37°C. The zones formed at the end of the incubation period were measured and evaluated. This assay was performed according to Strompfova *et al*.[13].

3. Results and Discussion

Antibiotics susceptibility

The resistance of *Enterococcus* toward antibiotics is well-documented, with a growing concern over the past decadesdue to endemic or epidemic rates observedin North America and the increasing levels of Europe [14]. Antibiotics susceptibility of *Enterococcus spp.*isolated from milk and some dairy products were studied. Tables (1 and 2) show that the studied antibiotics (n=7) were amikacin, azlocillin, cefotaxime, cefoxitin, cloxacillin, colistin, andlevofloxacin. The results presented here indicate that the studied Egyptian milk and cheeses could not be ruled out as a potential source for spreading antibiotic–resistance strains. Table (2) shows that all the 23 Enterococcus species strains were sensitive to amikacin, cefotaxime, and cefoxitin. Also, out of the 23*Enterococcus* species strains, 19 strains were sensitive to azlocillinand cloxacillin. In comparison, levofloxacin was susceptible to 21 strains. El - Shafei *et al*.[15] stated that all tested *E. faecium*strains (n=24) were sensitive to cophezalin while 20 strains to chloramphenicol, rimictan, and garamycin, 19 strains to ampicillin, 18 strains to erythromycin, 16 strains to carbenicillin, and 14 strains to sptrin.

Out of the 12*E. faecium* strains, Table (1) shows that only twostrains were resistant to azlocillin, cloxacillin, and levofloxacin each. In addition, none of the 12 *E. faecium* strains were resistant to amikacin, cefotaxime, and cefoxitin. Results also showed that most 12 *E. faecium* strains were sensitive to all studied antibiotics except colistin, whereas 12 *E. faecium* strains were resistant to this antibiotic. Also, 10 *E. durans* strains and 1 *E. faecalis* strain were resistant to colistin.

Table 1.Antibiotics are susceptiblepatterns of *Enterococcus* species isolated from milk and somedairy products.

Antibiotics	*E.faecium* (12 strains)			*E.durans* (10 strains)			*E.faecalis*(1 strain)		
	R	I	S	R	I	S	R	I	S
Amikacin	0	0	12	0	0	10	0	0	1
Azlocillin	2	2	8	2	1	7	0	0	1
Cefotaxime	0	0	12	0	0	10	0	0	1
Cefoxitin	0	0	12	0	0	10	0	0	1
Cloxacillin	2	4	6	2	4	4	0	1	0
Colistin	12	0	0	10	0	0	1	0	0
Levofloxacin	2	3	7	0	2	8	0	1	0

R: resistant; I: intermediate; S: sensitive.

Similarly, none of the 10 *E. durans* strains (Table 1) were resistant to antibiotics studied, namely, amikacin, cefotaxime, cefoxitin, and levofloxacin. Results also showed that most 10 *E. durans* strains were sensitive to all studied antibiotics. Also, the *E. faecalis* strain was susceptible to all studied antibiotics. Dabiza *et al*.[16] stated that strains of *Ent. faecium* showed resistance to antibiotics tested were garamycin (41.3%), ampicillin (26%), chloramphenicol, and erythromycin (23.9%). An earlier study by Batish and Ranganathan [17]showed that among 224 enterococcal isolated from samples of milk and milk products, 80 to 90% of these microorganisms were foundas sensitive or partially sensitive to chloramphenicol, erythromycin, kanamycin, and tetracycline. More recently, Giraffa *et al*.[18] reported that 75 enterococcal strains extracted from dairy sources exhibited an overall sensitivity (about 90 to 100% of all strains) to many antibiotics, particularly chloraphenicol ampicillin and novobiocin.

On the other hand, Cariolato *et al*.[11] stated that tested dairy strains showed very high percentages of susceptibility to most of the antibiotics tested, except for tetracycline, streptomycin, and erythromycin resistance which were detected in 30.8%, 25.6 %, and 17.9% of the strains, respectively.

In general, the most effective antibiotics against studied *Enterococcus* spp. were amikacin, cefotaxime, and cefoxitin 23 (100%), 23(100%) and 23 (100%) out of 23 *Enterococcus* species, azlocillin16 (69.6%) strains followed by levofloxacin15 (65.2%) and cloxacillin 10 (43.5%) strains, respectively. Also, all studied *Enterococcus spp.*showed a higher degree of resistance against colistin23 (100%)also, 4 (17.4%), 4 (17.4%), and 2 (8.7%) were resistant to azlocillin, cloxacillin, and levofloxacin, respectively (Table2).

Table 2. Number and percentage of susceptible(S), intermediate (I), and resistant(R)*Enterococcus* species against some antibiotics.

	R		I		S	
	No	%	No	%	No	%
Amikacin	0	0.0	0	0.0	23	100
Azlocillin	4	17.4	3	13.0	16	69.6
Cefotaxime	0	0.0	0	0.0	23	100
Cefoxitin	0	0.0	0	0.0	23	100
Cloxacillin	4	17.4	9	39.1	10	43.5
Colistin	23	100	0	0.0	0	0.0
Levofloxacin	2	8.7	6	26.1	15	65.2

R= Resistant (inhibition zone = 6 mm),I=intermediate sensitivity (inhibition zone > 6 –15 mm), S= susceptible (inhibition zone > 15 mm)

Cariolato *et al*.[11] found in their study that *Ent. faecalis* strains were mainly resistant to tetracycline (65.8 %) followed by streptomycin (42.1%), erythromycin (28.9%), norfloxacin (21.1%), chloramphenicol (18.4%), and gentamycin (10.5%). However, the results of this study showed a similar trend with streptomycin and erythromycin. Koluman *et al*. [20] studied 50 samples of cheese and 20 samples of Yogurt and stated that the highest resistance was recorded for a cream sample with the resistance of 12 types of antibiotics. They also said that 33 of 50 cheese samples (64%) were resist to at least three antibiotics and the highest resistance was recorded in cream cheese sample with resistance to 12 types of antibiotics. None of the isolated samples (n=70) from Turkish dairy products resisted vancomycin [19]. However, Upadhyaya *et al*. [20] stated that there had been an increase in the number of VRE in recent times.

The lower occurrence of dairy enterococci isolates resistant to clinically relevant antibiotics was studied as shown from our results and previous studies by Mannu *et al*., [21] and Cariolato *et al*., [11],indicating the low diffusion of antibiotic-resistant among food enterococci from milk and dairy products.

Bacteriocin production

Bacteriocinribosomal synthesized peptides with antimicrobial properties are produced by many living organisms ranging from prokaryotes to higher eukaryotes [22].Twenty-three *Enterococcus* species strains isolatedfrom Raw milk, Karish cheese, Domiati cheese, and Ras cheese were subjected to determine their ability to produce bacteriocin against indicator strains *B. cereus*,*B. thuringiensis*, *Staph. aureus*, *Str. pyogenes*, *E. coli* O157:H7,*S. typhimurium*, *Y. enterocolitica*and *P. aeruginosa*. All 23 *Enterococcus* species strains exhibited antimicrobial activity against most tested gram-positive and gram-negative foodborne pathogens and contaminant bacteria (Tables 3, 4, and 5).

Table 3.Bacteriocin production of *Enterococcus* species isolated from Raw milk.

Indicator strains	*Enterococcus* species (n=7)							Total		
	E. faecium Rm4-1	*E. faecium* Rm4-2	*E. faecium* Rm5-1	*E. faecium* Rm6-1	*E. durans* Rm1-2	*E. durans* Rm3-1	*E. durans* Rm3-2			
	Inhibition zone/mm							R	I	S
B. cereus	6	6	12	15	6	6	10	4	3	0
B. thuringiensis	12	12	16	18	12	12	16	0	4	3
Staph. aureus	6	6	14	16	6	13	6	4	2	1
Str. pyogenes	6	6	18	18	6	12	12	3	2	2
E. coli O157:H7	6	6	12	12	6	6	6	5	2	0
S. typhimurium	6	6	14	14	6	6	6	5	2	0
Y. enterocolitica	6	6	6	6	6	6	6	7	0	0
Ps. aeruginosa	6	6	16	18	6	12	13	3	2	2

R= Resistant (inhibition zone = 6 mm),I=intermediate sensitivity (inhibition zone >6 –15 mm), S= susceptible (inhibition zone > 15 mm)

The powerful bacteriocin (inhibition zone >15 mm) producers were observed with *E. faecium*Rm5-1against *B. thuringiensis*, *Str. pyogenes* and *Ps. aeruginosa*, *E. faecium* Rm6-1 against *B. thuringiensis*,*Staph. aureus*, *Str. pyogenes* and *Ps. aeruginosa*, *E.durans*Rm3-2 against *B. thuringiensis*, *E. faecium* Kc3-2 against *B. thuringiensis*, *E.durans*Kc1-1 against *B. thuringiensis*, *Staph. aureus* and *Ps. aeruginosa*, *E.durans*Kc5-2 against *B. cereus*,*Staph. aureus*,*B. thuringiensis*,*Str. pyogenes* and *Ps. aeruginosa*, *E.durans*Kc10-1 against *Ps. aeruginosa*,*E. faecalis* Kc2-1 against *Str. pyogenes*, *E. faecium* Dc1-1 against *B. thuringiensis*, *E. faecium* Dc10-2 against *B. cereus*, *Str. pyogenes*,*Ps. aeruginosa* and *B. thuringiensis*, *E. faecium* Rc3-1 against *Ps.*

aeruginosa and*E. durans*Rc3-2 against *B. thuringiensis*, *Staph. aureus*, *Str. pyogenes* and *Ps. aeruginosa*.

Yerlikaya and Akbulut [23]studied microorganisms isolated from milk, and dairy products found that*Enterococcus durans* 61E5 was the strain with the highest antimicrobial activity against *A. hydrophlia*, followed by 61E2. Meanwhile, some strains did not show activity against test bacteria, including *S. aureus, B. cereus*, *L. monocytogenes*, *P. aureginosa*, and *A. hydrophilia* [23]. Generally, most strains showed an effect on *E. coli*, followed by *L. monocytogenes* and *S. aureus*. All the strains except for *E. faecium*

Table 4.Bacteriocin production of *Enterococcus* species isolated from Karish cheese.

Indicator strains	*Enterococcus* species (n=8)								Total		
	E. faecium Kc1-2	*E. faecium* Kc3-2	*E. durans* Kc1 1	*E. durans* Kc4 2	*E. durans* Kc5 1	*E. durans* Kc5 2	*E. durans* Kc10-1	*E. faecalis* Kc2-1			
	Inhibition zone/mm								R	I	S
B. cereus	6	10	12	6	6	18	6	6	5	2	1
B. thuringiensis	12	16	18	12	12	22	12	12	0	5	3
Staph. aureus	6	6	16	6	6	16	6	12	5	1	2
Str. pyogenes	6	12	14	6	6	22	14	16	3	3	2
E. coli O157:H7	6	6	12	6	6	14	6	6	6	2	0
S. typhimurium	6	6	10	6	6	12	6	6	6	2	0
Y. enterocolitica	6	6	6	6	6	6	6	6	8	0	0
Ps. aeruginosa	6	6	18	6	6	20	16	14	4	1	3

R= Resistant (inhibition zone = 6 mm),I=intermediate sensitivity (inhibition zone >6 –15 mm), S= susceptible (inhibition zone > 15 mm)

Table 5.Bacteriocin production of *Enterococcus* species isolated from Domiati and Ras cheese.

Indicator strains	*Enterococcus* species (n=8)								Total		
	E. faecium Dc1-1	*E. faecium* Dc4-1	*E. faecium* Dc5-1	*E. faecium* Dc10-2	*E. durans* Dc9 2	*E. faecium* Rc2-1	*E. faecium* Rc3-1	*E. durans* Rc3 2			
	Inhibition zone/mm								R	I	S
B. cereus	12	6	6	18	6	6	6	14	5	2	1
B. thuringiensis	18	14	14	22	12	12	12	18	0	5	3
Staph. aureus	13	6	6	15	6	6	6	16	5	2	1
Str. pyogenes	14	6	6	16	6	14	14	22	3	3	2
E. coli O157:H7	12	6	6	14	6	6	6	14	5	3	0
S. typhimurium	6	6	6	12	6	6	6	15	6	2	0
Y. enterocolitica	6	6	6	6	6	6	6	6	8	0	0
Ps. aeruginosa	14	6	6	16	6	15	16	18	3	2	3

R= Resistant (inhibition zone = 6 mm),I=intermediate sensitivity (inhibition zone >6 –15 mm), S= susceptible (inhibition zone > 15 mm)

K1E3, *E. faecium* 67E3, and *E. durans* 79E3 had an antibacterial effect on *E. coli*. A very few strains were effective on *P. aureginosa*, *C. jejuni*, and *B. cereus*. It has been reported that *Enterococcus* strains isolated from various sources showed lower activity against *B. cereus*, whereas they were more effective against *E. coli* and *L. monocytogenes*[24,25,26].

Bacteriocin produced by enterococci proved to have attractive technological potential. Almost all of them are intensely active against food spoilers and foodborne pathogens such as *L.monocytogenes, Clostridium spp, Staph. aureus, Bacillus spp, Brochothrix spp, Vibrio cholerae* and *spoilage LAB*[27,28, 29,30,1].

Results of Table (6) show that out of 23 *Enterococcus species* strains, only 2 (8.7%), 9 (39.1%), 4 (17.4%), 6 (26.1%), and 8 (34.8%) showedpotentantimicrobial activity against indicator organisms namely, *B. cereus*, *B. thuringiensis*, *Staph. aureus*, *Str. pyogenes* and *Ps. aeruginosa*, respectively. Also, 7 (30.4%), 14 (60.9%), 5 (21.7%), 8 (34.8%), 7 (30.4%), 6 (26.1%) and 5 (21.7%)showedintermediateantimicrobial activity against indicator organisms namely, *B. cereus*, *B. thuringiensis*, *Staph. aureus*, *Str. pyogenes*, *E. coli* O157:H7, *S. typhimurium*,and *Ps. aeruginosa*, respectively. Similarly, the bacteriocin production rate was highest among the cream samples (33.51%), followed by Dahi samples (15.55%), raw milk samples (9.51%), cheddar cheese samples (9.2%), and human feces (4.22%)[31].Ghrairi *et al*. [32] stated that bacteriocin produced by *E. faecium* MMT21 was thermostable and thus could be useful as a food preservative, especially during food processing procedures involving a heating step. It was also found stable over a wide range of pH, which allows its application in acid as well as non-acid foods. Like most bacteriocins [33, 34, 35], *E. faecium* MMT21 bacteriocin was produced during the exponential growth phase, with the highest rate occurring during the beginning of the stationary phase.On the other hand, 14 (60.9%), 14 (60.9%),9 (39.1%),16 (69.6%), 17 (73.9%), 23 (100%) and 10 (43.5%) showed no inhibition zones against tested indicator organism's *B. cereus*, *Staph. aureus*, *Str. pyogenes*, *E. coli* O157:H7, *S. typhimurium*, *Y. enterocolitica*,and *Ps. aeruginosa*, respectively.

Table 6.Number and percentage of susceptible (S), intermediate (I), and resistant (R) *Enterococcus* species against some pathogenic and spoilage bacteria.

Indicator strains	Reactions					
	R		I		S	
	No	%	No	%	No	%
B. cereus	14	60.9	7	30.4	2	8.7
B. thuringiensis	0	0.0	14	60.9	9	39.1
Staph. aureus	14	60.9	5	21.7	4	17.4
Str. pyogenes	9	39.1	8	34.8	6	26.1
E. coli O157:H7	16	69.6	7	30.4	0	0.0
S. typhimurium	17	73.9	6	26.1	0	0.0
Y. enterocolitica	23	100	0	0.0	0	0.0
Ps. aeruginosa	10	43.5	5	21.7	8	34.8

R= Resistant (inhibition zone = 6 mm) I=intermediate sensitivity (inhibition zone > 6 –15 mm)
S= susceptible (inhibition zone > 15 mm)

Gupta and Malik[31] examined sixty potent bacteriocin-producing isolates of enterococci for the incidence of virulence factors for their safe exploitation. They found none of the isolates exhibited gelatinase or hemolysis activity. However, they stated that only four bacteriocin-producing isolates among 60 were observed to be vancomycin-resistant. It is well known that bacteriocinogenic strains present a potential for food preservation, pathogen control, or even inhibition of biofilm formation [36,37,38, 39].*Enterococcus spp*. has been reported to produce bacteriocins, which inhibited Gram-positive foodborne bacteria and intestinal pathogens [40,41,15].

Bacteriocin-like antagonism displayed by enterococci was first reported by Kjems[42]. In recent years, several *E. faecium* and *E. faecalis* strains from dairy products [43,44] displaying antilisterial activity have been isolated, which makes them potent important in food preservation. The antimicrobial effects of a broad spectrum of substances from *E. faecium* proved effective against Gram-negative and Gram-positive bacteria [45].

One of the main drawbacks of many antimicrobial agents is their ability to inhibit the undesirable bacteria and lactic starter and nonstarter culture when preserving fermented foods. In this study, our antimicrobial producer enterococci may be of interest because of their spectrum of activity, as it would allow relatively selective inhibition of foodborne pathogens.

Conclusion

Enterococcus species strains isolated from raw milk and some cheese samples were tested and foundinhibitory against some Gram-positive and Gram-negative foodborne pathogens and contaminating bacteria. According to the antimicrobial activity of Enterococcus species proved during the current study, they could be applied as bio preservatives in fermented foods such as cheese and sausages in place of using chemical preservatives. On the other hand, harmless enterococci exhibiting antagonistic effects against pathogenic bacteria used as bio preservatives may also extend the shelf life of food products cause of inhibiting the growth of spoilage and pathogenic bacteria with a subsequent reduction in the use of antibiotics.

Conflicts of interest

There are no conflicts to declare.

Formatting of funding sources

No funding source to declare

References

[1] Foulquie Moreno, M. R., Sarantinopoulos, P., Tsakalido, E. and De Vyst, L., The role and application of enterococci in food and health. International Journal of Food Microbiology **106**, 1 – 24 (2006). https://doi.org/10.1016/j.ijfoodmicro.2005.06.026

[2] Hanchi, H., Mottawea, W., Sebei, K. and Hammami, R., The genus *Enterococcus*: between probiotic potential and safety concerns—an update. Frontiers in microbiology, **9**, 1791(2018). https://doi.org/10.3389/fmicb.2018.01791

[3] Klein, G., Taxonomy, ecology and antibiotic resistance of enterococci from food and the gastrointestinal tract. International journal of food microbiology, **88**, 123-131(2003). doi: 10.1016/s0168-1605(03)00175-2

[4] Chen, X., Song, Y. Q., Xu, H. Y., Menghe, B. L. G., Zhang, H. P. and Sun, Z. H., Genetic relationships among *Enterococcus faecalis* isolates from different sources as revealed by multilocus sequence typing. Journal of dairy science, 98(8), 5183-5193 (2015). http://dx.doi.org/10.3168/jds.2015-9571

[5] Robinson, R. K., Dairy microbiology handbook: the microbiology of milk and milk products. John Wiley & Sons (2005).

[6] Franz, M.A.P., Stiles, M., Schleifer, K. and Holzapfel, W. H., Enterococci in foods – a conundrum for food safety. International Journal of Food Microbiology,**88**,105 – 122 (2003). https://doi.org/10.1016/S0168-1605(03)00174-0

[7] Franz, C. M., Huch, M., Abriouel, H., Holzapfel, W. and Gálvez, A., Enterococci as probiotics and their implications in food safety. International journal of food microbiology, **151**, 125-140 (2011). doi: 10.1016/j.ijfoodmicro.2011.08.014.

[8] Floriano, B., Ruiz-Barba, J. L. and Jimenez-Diaz, R., Purification and genetic characterization of enterocin I from *Enterococcus faecium* 6T1a, a novel antilisterial plasmid-encoded bacteriocin which does not belong to the pediocin family of bacteriocins. Applied and Environmental Microbiology, **64**, 4883 – 4890 (1998). doi: 10.1128/AEM.64.12.4883-4890.1998.

[9] Franz, C. M. A. P., Holzapfel, W. H. and Stiles, M. E., Enterococci at the crossroads of food safety? International Journal of Food Microbiology, **47**, 1 – 24 (1999). https://doi.org/10.1016/S0168-1605(99)00007-0

[10] Gelsomino, R., Vancanneyt, M., Condon, S., Swings, J. and Cogan, T. M., Enterococcal diversity in the environment of an Irish Cheddar-type cheese making factory. International journal of food microbiology, **71**, 177-188 (2001). https://doi.org/10.1016/S0168-1605(01)00620-1

[11] Cariolato, D., Andrighetto, C. and Lombardi, A., Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. Food Control, **19**, 886 – 892 (2008). https://doi.org/10.1016/j.foodcont.2007.08.019

[12] Baumgartner, A., Kueffer, M. and Rohner, P., Occurrence and antibiotic resistance of enterococci

in various ready-to-eat foods. Archive Fur Lebensmittelhygiene, 52, 16 – 19 (2001).

[13] Strompfova, V., Laukoua, A., Simonova, M. and Marcinakova, M., Occurrence of the structural enterocin A, P, B, L50B genes in enterococci of different origin. Veterinary Microbiology, **132**, 293 – 301 (2008).
https://doi.org/10.1016/j.vetmic.2008.05.001

[14] Mutnick, A. H., Biendenbach, D. J. and Jones, R. N., Geographic variations and trends in antimicrobial resistance among *Enterococcus faecalis* and *Enterococcus faecium* in the SENTRY antimicrobial surveillance program (1997 – 2000). Diagn Microbiol. Infect. Dis., **46**, 63 – 68 (2003).
https://doi.org/10.1016/S0732-8893(03)00012-9

[15] El – Shafei, K., Ibrahim, G. A. and Tawfik, N. F., Beneficial uses of locally isolated lactic acid bacteria. Egyptian J. Dairy Sci., **30**, 15 – 25 (2002).

[16] Dabiza, N. M. A., Effat, B. A. and Sharaf, O.M., The antibacterial effect of probiotic bacteria isolated from some dairy products. Egyptian J. Dairy Sci., **33**, 49 – 64 (2005).

[17] Batish, U. K. and Ranganathan, B., Antibiotic susceptibility of dexoyribonuclease – positive enterococci isolated from milk and milk products and their epidemiological significance. International Journal of Food Microbiology, **3**, 331-342 (1986).
https://doi.org/10.1016/0168-1605(86)90015-2

[18] Giraffa, G., Carminati, D. and Neviani, E., Enterococci isolated from dairy products: A review of risks and potential technological use. Journal of Food Protection, **60**,732 – 738 (1997).
https://doi.org/10.1016/0168-1605(86)90015-2

[19] Koluman, A., Akan, L. S. and Cakiroglu, F. P., Occurrence and antimicrobial resistance of enterococci in retail foods. Food Control, **20**, 281 – 283 (2009).
https://doi.org/10.1016/j.foodcont.2008.05.007

[20] Upadhyaya, P. M. G., Ravikumar, K. L. and Umapathy, B. L., Review of virulence factors of *Enterococcus*: An emerging nosocomial pathogen. Indian Journal of Medical Microbiology, **27**, 301 – 305 (2009).
https://doi.org/10.4103/0255-0857.55437

[21] Mannu, L., Paba, A., Daga, E., Comunian, R., Zanetti, S., Dupre, I. and Sechi, L. A., Comparison of the incidence of virulence determinants and antibiotic resistance between *Enterococcus faecium* strains of dairy, animal and clinical origin. International Journal of Food Microbiology, **88**, 291 – 304 (2003).
https://doi.org/10.1016/S0168-1605(03)00191-0

[22] Papagianni, M., Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function and applications. Biotechnology Advances, **21**, 465–499 (2003).
https://doi.org/10.1016/S0734-9750(03)00077-6

[23] Yerlikaya, O. and Akbulut, N., In vitro characterisation of probiotic properties of *Enterococcus faecium* and *Enterococcus durans* strains isolated from raw milk and traditional dairy products. International Journal of Dairy Technology, **73**, 98-107 (2020).
https://doi.org/10.1111/1471-0307.12645

[24] Hajikhani, R., Beyatli, Y. and Aslim, B., Antimicrobial activity of enterococci strains isolated from white cheese. International journal of dairy technology, **60**, 105-108 (2007).
https://doi.org/10.1111/j.1471-0307.2007.00304.x

[25] Nascimento, M. D. S. D., Moreno, I. and Kuaye, A. Y., Antimicrobial activity of *Enterococcus faecium* FAIR-E 198 against gram-positive pathogens. Brazilian Journal of Microbiology, **41**, 74-81 (2010).
doi: 10.1590/S1517-83822010000100013

[26] Lertworapreecha, N., Poonsuk, K. and Chalermchiakit, T., Selection of potential *Enterococcus faecium* isolated from Thai native chicken for probiotic use according to the in vitro properties. Songklanakarin Journal of Science and Technology, **33**, 9-14 (2011).
DOI: 10.1007/s00203-021-02273-y

[27] Sabia, C., Manicardi, G., Messi, P., de Niederhausern, S. and Bondi, M., Enterocin 416K1, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416K1 isolated from Italian sausages. Int. J. Food Microbiology, **75**, 163 – 170 (2002).
https://doi.org/10.1016/S0168-1605(01)00741-3

[28] Leroy, F. and De Vuyst, L., Bacteriocin production by *Enterococcus faecium* RZS C5 is cell density limited and occurs in the very early growth phase. International Journal of Food Microbiology, **72**, 155 – 164 (2002).
https://doi.org/10.1016/S0168-1605(01)00635-3

[29] Leroy, F., Moreno, M. F. and De Vuyst, L., *Enterococcus faecium* RZS C5, an interesting bacteriocin producer to be used as a co-culture in food fermentation. International journal of food microbiology, **88**, 235-240 (2003).
DOI: 10.1016/s0168-1605(03)00185-5

[30] Garcia, M. T., Canamero, M. M., Lucas, R., Omar, N. B., Pulido, R. P. and Galvez, A., Inhibition of *Listeria monocytogenes* by enterocin EJ97 produced by *Enterococcus faecalis* EJ97.

International Journal of Food Microbiology, **90**, 161 – 170 (2004). https://doi.org/10.1016/S0168-1605(03)00051-5

[31] Gupta, H. and Malik, R. K., Incidence of virulence in bacteriocin – producing enterococcal isolates. Lait, **87**, 587 – 601 (2007). https://doi.org/10.1051/lait:2007031.

[32] Ghrairi, T., Frere, J., Berjeaud, J. M. and Manai, M., Purification and characterization of bacteriocins produced by *Enterococcus faecium* from Tunisian rigouta cheese. Food Control, **19**, 162 – 169 (2008). https://doi.org/10.1016/j.foodcont.2007.03.003

[33] Camposa, C. A., Rodriguez, O., Calo-Mata, P., Prado, M. and Barros-Velazquez, J., Preliminary characterization of bacteriocins from *Lactococcus lactis, Enterococcus faecium* and *Enterococcus mundtii* strains isolated from turbot (Psetta maxima). Food Research International, **39**, 356 – 364 (2006). https://doi.org/10.1016/j.foodres.2005.08.008

[34] Parente, E., Brienza, C., Ricciardi, A. and Addario, G., Growth and bacteriocin production by *Enterococcus faecium* DPC1146 in batch and continuous culture. Journal of Industrial Microbiology and Biotechnology, **18**, 62 – 67 (1997). https://doi.org/10.1038/sj.jim.2900368

[35] Todorov, S. D. and Dicks, L. M. T., Screening for bacteriocin producing lactic acid bacteria from boza, a traditional cereal beverage from Bulgaria comparison of the bacteriocins. Process Biochemistry, **41**, 11 – 19 (2006). https://doi.org/10.1016/j.procbio.2005.01.026

[36] Sulzer, G. and Busse, M., Growth inhibition of *listeria* spp. on camembert cheese by bacteria producing inhibitory substance. International Journal of Food Microbiology, **14**, 287 – 296 (1991).https://doi.org/10.1016/0168-1605(91)90120-E

[37] Franz, C. M., Grube, A., Herrmann, A., Abriouel, H., Starke, J., Lombardi, A., Tauscher, B. and Holzapfel, W. H., Biochemical and genetic characterization of the two – peptide bacteriocin enterocin 1071 produced by *Enterococcus faecalis* FAIR – E 309. Applied and Environmental Microbiology, **68**, 2550 – 2554 (2002). DOI: 10.1128/AEM.68.5.2550-2554.2002.

[38] Giraffa, G., Functionality of enterococci in dairy products. International Journal of Food Microbiology, **88**, 215 – 222 (2003). https://doi.org/10.3168/jds.S0022-0302(94)77055-7

[39] Minei, C. C., Gomes, B. C., Ratti, R. P., DAngelis, C. E. M. and De Martinis, E. C. P., Influence of peroxyacetic acid, nisin and co-culture with *Enterococcus faecium* on *Listeria monocytogenes* biofilm formation. J. Food Prot., **71**, 634 – 638 (2008). https://doi.org/10.4315/0362-028X-71.3.634

[40] Franz, C. M. A. P., Schillinger, U. and Holzapfel, W. H., Production and characterization of enterocin 900, a bacteriocin produced by *Enterococcus faecium* BFE 900 from black olives. International journal of food microbiology, **29**, 255-270 (1996). https://doi.org/10.1016/0168-1605(95)00036-4

[41] Du Toit, M., Franz, C. M. A. P., Dicks, L. M. T. and Holzapfel, W. H., Preliminary characterization of bacteriocins produced by *Enterococcus faecium* and *Enterococcus faecalis* isolated from pig faeces. Journal of Applied Microbiology, **88**, 482-494 (2000). https://doi.org/10.1046/j.1365-2672.2000.00986.x

[42] Kjems, E., Studies on streptococcal bacteriophages: I. Techniques for isolating phage producing strains. Pathology and Microbiology Scandinavia, **36**, 433 – 440 (1995). https://www.cabdirect.org/cabdirect/abstract/19552703156.

[43] McKay, A. M., Antimicrobial activity of *Enterococcus faecium* against *Listeria* spp. Lett. Applied Microbiology, **11**, 15 – 17 (1990). https://doi.org/10.1111/j.1472-765X.1990.tb00125.x

[44] Giraffa, G., Neviani, E. and Torri Tarelli, G., Antilisterial activity by enterococci in a model predicting the temperature evolution of Taleggio, an Italian soft cheese. Journal of Dairy Science, **77**, 1176 – 1182 (1994). https://doi.org/10.3168/jds.S0022-0302(94)77055-7

[45] Laukova, A., Marekova, M. and Javorsky, P., Detection and antimicrobial spectrum of a bacteriocin-like substance produced by *Enterococcus faecium* CCM4231. Letters in Applied Microbiology, **16**, 257-260 (1993). https://doi.org/10.1111/j.1472-765X.1993.tb01413.x