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SAFETY ASSESSMENT AND ANTIMICROBIAL POTENTIAL OF *Enterococcus* spp. ISOLATED FROM RAW GOAT MILK AND CHEESE

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ABSTRACT: This study aimed to assess the safety and antimicrobial properties of *Enterococcus* species isolated from raw goat milk and cheese. The isolates were evaluated for hemolytic activity on blood agar plates and antibiotic susceptibility by determining the minimum inhibitory concentrations (MICs) of five antibiotics: ampicillin, tetracycline, gentamicin, streptomycin, and vancomycin. Furthermore, the antagonistic activity of the *Enterococcus* strains against indicator bacteria was assessed using the agar-well diffusion method. Hemolytic activity results demonstrated α-hemolysis in all isolates. Among the 71 *Enterococcus* isolates examined, 32.39% exhibited resistance to at least one antibiotic, with resistance profiles varying between species. *Enterococcus faecalis* isolates predominantly showed resistance to vancomycin, ampicillin, tetracycline, and gentamicin, while *Enterococcus faecium* and *Enterococcus hirae* displayed resistance to vancomycin with different susceptibility patterns to the other antibiotics. The antagonistic assays revealed a broad spectrum of inhibitory effects, with variation in inhibition zone diameters. These findings provide valuable insights into the safety and antimicrobial potential of *Enterococcus* spp. from raw goat milk and cheese and give an insight into their potential applications in the food industry.

Keywords: Enterococci, antibiotic sensitivity, hemolytic activity, antimicrobial activity, goat cheese

INTRODUCTION

The quality, production and preservation of dairy products depends on a diverse group of lactic acid bacteria (GRUJOVIĆ *et al*., 2022). Bacteria from the genus *Lactobacillus* have long been used in the preservation of dairy products, while bacteria from the genus *Enterococcus* have not yet received GRAS (Generally Recognized As Safe) status, although they represent the autochthonous microbiota of cheeses (COELHO *et al*., 2022). Additionally, enterococci are

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known for their ability to develop resistance to antibiotics, posing challenges in both clinical and food production settings (GRUJOVIĆ *et al*., 2022). An additional critical aspect of its pathogenic potential is its ability to transfer antibiotic resistance genes to other foodborne pathogens, such as *Listeria monocytogenes*, posing significant risks to consumer health (JAHAN and HOLLEY, 2016).

Among the many *Enterococcus* species, *Enerococcus faecium* and *Enterococcus faecalis* emerge as the most frequently isolated ones within the food industry (GOMES *et al*., 2008; GRUJOVIĆ *et al*., 2019). Despite concerns regarding their safety due to their association with nosocomial infections such as endocarditis, bacteremia, and urinary tract infections, enterococci demonstrate beneficial roles in cheese and meat production as starter or probiotic cultures (FURLANETO-MAIA *et al*., 2014). Their presence in dairy products is particularly noteworthy for their contributions to ripening and flavor development, likely facilitated through mechanisms such as proteolysis, lipolysis, exopolysaccharide production, and diacetyl production via citrate metabolism (ABOUELNAGA *et al*., 2016). Additionally, certain enterococcal strains, notably *Ent. faecalis* and *Ent. faecium*, produce bacteriocins - potent inhibitory substances capable of combating foodborne pathogens and spoilage microorganisms such as *Listeria monocytogenes* and *Staphylococcus aureus*, including both vegetative cells and spores (RAAFAT *et al*., 2016). However, the potential risks associated with enterococci as starter cultures cannot be ignored, given their propensity to cause infections (FURLANETO-MAIA *et al*., 2014; SANLIBABA and SENTURK, 2018).

In recent years, there has been increasing interest in understanding the safety and potential beneficial properties of enterococci isolated from food sources, including raw goat milk and cheese. This study aims to evaluate the safety of *Enterococcus* species isolated from raw goat milk and cheese through a comprehensive safety evaluation, including tests for hemolytic activity and antibiotic susceptibility. Furthermore, the antagonistic potential of these isolates against common foodborne pathogens will be investigated, shedding light on their potential use as natural antimicrobial agents in food preservation. Understanding the safety and antagonistic potential of enterococci isolated from raw goat milk and cheese is essential for assessing their suitability for use in food production and ensuring consumer safety**.**

MATERIAL AND METHODS

The cheese manufacture and sampling were described in detail by MLADENOVIĆ *et al*. (2022) and GRUJOVIĆ *et al*. (2024). Briefly, the cheese being investigated was produced in the spring of 2021 in Pajsijević village, Central Serbia. Fresh goat milk with a pH of 6.6 was collected, filtered, and heated to 32°C before adding liquid rennet. No bacterial cultures were used. The coagulated mass was cut into cubes, agitated, and left to drain. Salt was applied, and the cheese cubes were submerged in a brine solution, and then aged in a cellar at 15-16°C for 28 days. Samples were collected at 0, 7, 14, 21, and 28 days for microbiological analysis. The samples were kept at 4°C and analyzed within 24 hours. Raw goat milk was also examined.

Tested strains

The methodology for isolating and characterizing bacteria, as outlined in GRUJOVIĆ *et al.* (2024), involved a comprehensive process. Briefly, a 200 g composite goat cheese sample was collected using sterile techniques, and 10 g was homogenized in 90 mL of 2% sodium citrate solution (pH 7.5) heated to 45°C. Successive 10-fold dilutions (up to 10^{-7}) were prepared. For microbiological analysis, 1 mL of each dilution and fresh goat milk was plated on bile esculin agar (BEA) and incubated for 72 h at 32°C.

Single colonies from BEA plates were purified and subjected to microscopic examination, Gram staining, and catalase tests. Gram-positive, catalase-negative isolates were

further characterized using biochemical tests, including pH, temperature, NaCl tolerance, CO_2 production, and metabolic activity. Enterococci were identified using the Microgen Strep ID (Microgen Bioproducts, Camberley (Surrey), United Kingdom) system.

Isolates were preserved at −20°C and −80°C in glycerol-containing M17 broth and revitalized for testing. MALDI-TOF mass spectrometry was used for final identification, involving protein extraction and matrix-assisted laser desorption. Matching scores ≥2.00 confirmed species-level identification. The results obtained identified the following bacteria: *Ent. faecalis*, *Ent. faecium*, and *Ent. hirae*. The distribution of *Enterococcus* species isolated from raw goat milk and cheese during ripening is shown in Table 1. *Enterococcus faecalis* isolates from raw goat milk samples are labeled as M1–M8. For comparative purposes, *Enterococcus faecalis* ATCC 29211 was used.

Table 1. Distribution of *Enterococcus* species in goat milk and cheese during ripening (modified from GRUJOVIĆ *et al*., 2024)

Origin	Day of isolation	Species			Total number of isolates
		Ent. faecalis	*Ent. faecium*	*Ent. hirae*	
Goat milk	-	8	n.d.	n.d.	8
Goat cheese	0	11	n.d.	n.d.	11
	7^{th}	5	n.d.	n.d.	5
	14^{th}	4	3	n.d.	7
	21^{st}	6	6	2	14
	28^{th}	15	4	7	26
Total number of isolates		49	13	9	71

n.d. – not detected

Evaluation of the safety aspect of tested isolates

The safety aspect of the tested bacteria involved examining their hemolytic activity and resistance to selected antibiotics.

Hemolytic activity

To assess the safety aspect of the tested isolates, their ability to synthesize extracellular proteins, specifically hemolysins, on blood agar plates was investigated (BUXTON, 2005). Hemolytic activity was tested on sheep blood agar plates, incubated at 37°C for 24 hours. *Staphylococcus aureus* ATCC 25923 was used for quality control. The β-hemolytic reaction leads to complete lysis of erythrocyte cells, resulting in a clear halo around the colony, while the α-hemolytic reaction involves the appearance of a greenish color. A γ-hemolytic reaction indicates that the strain showed no hemolytic activity.

Resistance to antibiotics

The antibiotic sensitivity of isolated enterococci was investigated using the microdilution method with resazurin, and the minimum inhibitory concentration (MIC) was determined (SARKER *et al*., 2007). Ampicillin, tetracycline, gentamicin, streptomycin, and vancomycin (Sigma Chemicals Co., USA) were used in concentrations ranging from 0.05 to 4000 μg/ml for this study. The method was described in detail by GRUJOVIĆ *et al*. (2024).

Antagonistic potential

The antagonistic potential of isolated enterococci was screened using the agar-well diffusion method (TAGG AND MCGIVEN, 1971). Three standard strains, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, and *S. aureus* ATCC 25923, were employed, along with one strain isolated from the same cheese, *Escherichia coli* G14 (MLADENOVIĆ *et al*., 2022),

and a human isolate *Klebsiella pneumoniae* (generously provided by the Institute of Public Health Kragujevac), as indicator strains. The collection of identified bacterial species and ATCC stains was maintained in a 20% glycerol/medium mixture at -80°C. Before use, indicator bacteria were revitalized by two consecutive transfers in Nutrient agar (Torlak, Belgrade, Serbia) at 37°C.

Soft Nutrient agar (0.7%, w/v), containing indicator strains, was overlaid onto M17 plates. Wells were created in the lawn of hardened soft agars. 100 μL aliquots of supernatant from overnight cultures (18 h) centrifuged at 10.000 rpm for 30 min at 4°C, were adjusted to pH 6.5 by adding 12 M NaOH and were then filter-sterilized. The neutralized and filtered supernatant was placed in the wells (6 mm) and assayed for antagonistic activity against indicator strains. The plates were then incubated overnight at 37°C. A clear zone of inhibition around the well was measured, and the size of the well was subtracted from the total zone diameter to compensate for the background zone.

RESULTS AND DISCUSSION

Evaluation of the safety aspect of tested isolates

The safety of the isolates was studied by testing their hemolytic activity and antibiotic sensitivity. *Enterococcus* spp. showed α hemolysis. The minimum inhibitory concentration (MIC) of five antibiotics (ampicillin, tetracycline, gentamicin, streptomycin, and vancomycin) was determined for 71 *Enterococcus* strains (Table 2). The results were compared against resistance criteria by the European Food Safety Authority (EFSA). Table 3 shows the range of MIC values (μg/ml) for the tested isolates, while Table 4 shows the percentage of sensitive and resistant isolates among *Enterococcus* genera.

Among 71 *Enterococcus* isolates, 23 of them (32.39%) showed resistance to at least one tested antibiotic. Specifically, among *Ent. faecalis* isolates, the highest proportion exhibited resistance to vancomycin (isolates M-2, C0-4, C0-5, C0-11, C7-3, C7-5, C21-2, C21-6, C28-4, C28-11, and C28-3), followed by ampicillin (isolates M-3, C0-4, C0-11, C7-5, C21-2, C28-5, and C28-10), tetracycline (isolates C0-4, C7-3, C21-6, C28-6, and C28-13) and gentamycin (isolates M-3, M-7, C7-3, and C21-5). All tested isolates were sensitive to streptomycin. Isolates M-3, C0-11, C7-5, C21-2, C21-6, and C28-13 (12.24%) were resistant to two of five tested antibiotics while isolates C0-4 and C7-3 (4.08%) were resistant to three of five tested antibiotics.

Among *Ent. faecium* isolates, the highest proportion exhibited resistance to vancomycin (isolates C21-5, and C28-3), followed by ampicillin (isolate C21-5) and tetracycline (isolate C21-3). All tested isolates were sensitive to gentamycin and streptomycin. Isolate C21-5 was resistant to two of the five tested antibiotics.

Among *Ent. hirae* isolates, the highest proportion exhibited resistance to vancomycin (isolates C21-1, C28-3, and C28-5), followed by tetracycline (isolates C28-3 and C28-5) and ampicillin (isolate C28-5). All tested isolates were sensitive to gentamycin and streptomycin. Isolate C28-3 was resistant to two of five tested antibiotics, while isolate C28-5 was resistant to three of five tested antibiotics.

Table 2. Antibiotic sensitivity of isolated *Enterococcus* spp.

Species	Isolate	Ampicillin	Tetracycline	Gentamicin	Streptomycin	Vancomycin	Hemolysis
Ent. faecalis	M-1	1.56	0.78	12.5	75	2.34	α
Ent. faecalis	M-2	0.195	0.58	25	25	6.25	α
Ent. faecalis	M-3	2.34	1.56	50	25	3.12	α
Ent. faecalis	M-4	0.097	3.12	6.25	75	1.56	α
Ent. faecalis	M-5	1.56	0.39	12.5	50	3.12	α
Ent. faecalis	M-6	0.78	0.58	3.12	62.5	1.56	α
Ent. faecalis	M-7	1.56	2.34	50	100	0.78	α
Ent. faecalis	M-8	0.195	3.12	6.25	25	1.56	α
Ent. faecalis	C0-1	0.39	1.56	12.5	50	3.12	α
Ent. faecalis	C0-2	1.56	0.78	12.5	50	3.12	α
Ent. faecalis	C0-3	1.56	0.39	3.12	18.75	0.78	α
Ent. faecalis	C0-4	3.12	12.5	25	62.5	25	α
Ent. faecalis	C0-5	0.78	1.56	3.12	100	9.37	α
Ent. faecalis	C0-6	0.195	0.78	12.5	25	3.12	α
Ent. faecalis	C0-7	0.39	3.12	6.25	25	1.56	α
Ent. faecalis	C0-8	0.097	0.58	25	50	1.56	α
Ent. faecalis	C0-9	0.78	0.195	3.12	12.5	1.56	α
Ent. faecalis	C0-10	1.56	0.78	12.5	62.5	2.34	α
Ent. faecalis	C0-11	2.34	3.12	25	62.5	25	α
Ent. faecalis	C7-1	1.56	0.39	12.5	25	3.12	α
Ent. faecalis	C7-2	0.097	1.56	25	25	0.78	α
Ent. faecalis	C7-3	0.78	6.25	50	75	6.25	α
Ent. faecalis	C7-4	0.78	1.17	6.25	25	1.56	α
Ent. faecalis	C7-5	3.12	0.78	25	50	12.5	α

Table 2. continued							
Ent. faecalis	C14-1	0.195	3.12	12.5	25	1.56	α
Ent. faecalis	C14-2	0.39	3.12	25	18.75	0.78	α
Ent. faecalis	C14-3	1.56	1.17	25	50	3.12	α
Ent. faecalis	C14-4	0.097	0.39	3.12	50	1.56	α
Ent. faecalis	C21-1	0.097	3.12	6.25	25	0.78	α
Ent. faecalis	C21-2	2.34	1.56	12.5	50	6.25	α
Ent. faecalis	C21-3	0.78	1.56	25	62.5	3.12	α
Ent. faecalis	C21-4	0.097	0.78	3.12	18.75	2.34	α
Ent. faecalis	C21-5	0.58	0.39	50	75	1.56	α
Ent. faecalis	C21-6	1.56	6.25	3.12	50	12.5	α
Ent. faecalis	C28-1	0.39	1.56	12.5	25	0.78	α
Ent. faecalis	C28-2	0.39	1.56	25	62.5	3.12	α
Ent. faecalis	C28-3	0.195	3.12	25	18.75	2.34	α
Ent. faecalis	C28-4	0.58	0.78	3.12	100	6.25	α
Ent. faecalis	C28-5	2.34	0.78	6.25	25	3.12	α
Ent. faecalis	C28-6	0.78	12.5	25	50	1.56	α
Ent. faecalis	C28-7	0.39	0.195	12.5	25	3.12	α
Ent. faecalis	C28-8	0.58	0.39	6.25	25	3.12	α
Ent. faecalis	C28-9	0.097	3.12	25	18.75	1.56	α
Ent. faecalis	C28-10	3.12	0.78	25	62.5	1.56	α
Ent. faecalis	C28-11	0.195	1.17	12.5	75	6.25	α
Ent. faecalis	C28-12	0.78	1.56	3.12	62.5	2.34	α
Ent. faecalis	C28-13	0.58	6.25	12.5	50	9.37	α
Ent. faecalis	C28-14	1.56	0.39	1.56	50	3.12	α
Ent. faecalis	C28-15	0.097	3.12	25	100	3.12	α

Table 2. continued							
Ent. faecium	C14-1	0.78	0.195	12.5	25	2.34	α
Ent. faecium	C14-2	0.195	0.78	12.5	25	1.56	α
Ent. faecium	C14-3	0.39	1.56	3.12	25	0.78	α
Ent. faecium	C21-1	1.56	0.78	12.5	18.75	2.34	α
Ent. faecium	C21-2	1.56	0.39	6.25	62.5	3.12	α
Ent. faecium	C21-3	0.78	6.25	25	75	1.56	α
Ent. faecium	C21-4	0.195	1.56	3.12	12.5	0.78	α
Ent. faecium	C21-5	3.12	0.78	12.5	100	6.25	α
Ent. faecium	C21-6	0.78	3.12	6.25	25	1.56	α
Ent. faecium	C28-1	1.56	0.78	6.25	50	2.34	α
Ent. faecium	C28-2	0.097	0.39	1.56	62.5	3.12	α
Ent. faecium	C28-3	0.78	3.12	25	62.5	4.69	α
Ent. faecium	C28-4	0.39	1.56	6.25	18.75	1.56	α
Ent. hirae	C21-1	1.56	0.195	12.5	25	12.5	α
Ent. hirae	C21-2	0.097	3.12	1.56	100	2.34	α
Ent. hirae	C28-1	0.195	0.39	6.25	25	1.56	α
Ent. hirae	C28-2	1.56	1.17	25	25	2.34	α
Ent. hirae	C28-3	1.56	6.25	25	75	50	α
Ent. hirae	C28-4	0.39	0.78	12.5	50	1.56	α
Ent. hirae	C28-5	3.12	6.25	12.5	50	4.69	α
Ent. hirae	C28-6	0.195	3.12	25	75	1.56	α
Ent. hirae	C28-7	0.78	0.39	12.5	50	3.12	α
Ent. faecalis	ATCC 29211	1.56	8	n.d.	n.d.	250	β

The values represent MIC (minimum inhibitory concentrations) expressed in μg/mL

Table 3. The range of MIC values for the tested isolates

Species/antibiotics	Ampicillin	Tetracycline	Gentamicin	Streptomycin	Vancomycin
Ent. faecalis	0.097-3.12	0.39-12.5	1.56-50	18.75-100	0.78-25
Ent. faecium	0.195-3.12	0.195-6.25	1.56-25	12.5-100	0.78-6.25
Ent. hirae	0.097-3.12	0.195-6.25	1.56-25	25-100	1.56-50

The values represent MIC (minimum inhibitory concentrations) expressed in µg/ml

Table 4. The percentage of sensitive and resistant isolates among *Enterococcus* species

Species	***Ent. faecalis* (49 isolates)**		***Ent. faecium* (13 isolates)**		***Ent. hirae* (9 isolates)**	
Antibiotics/Interpretive criteria	Susceptible (%)	Resistant (%)	Susceptible (%)	Resistant (%)	Susceptible (%)	Resistant (%)
Ampiciline	42 (85.71%)	7 (14.29%)	12 (92.31%)	1 (7.69%)	8 (88.89%)	1 (11.11%)
Tetracycline	44 (89.8%)	5 (10.2%)	12 (92.31%)	1 (7.69%)	7 (77.78%)	2 (22.22%)
Gentamycin	45 (91.84%)	4 (8.16%)	13 (100%)	0	9 (100%)	0
Streptomycin	49 (100%)	0	13 (100%)	0	9 (100%)	0
Vancomycin	38 (77.55%)	11 (22.45%)	11 (84.62%)	2 (15.38%)	6 (66.67%)	3 (33.33%)

SANLIBABA and SENTURK (2018) documented that 9.9% of *Enterococcus* strains (21 out of 52) isolated from cheeses in Turkey exhibited γ-hemolytic characteristics, while the majority (78.8%, 168 out of 213) displayed α-hemolytic characteristics. This distribution of hemolytic types, with α-hemolysis being predominant, is consistent with findings from previous investigations by TUNCER (2008), BARBOSA *et al*. (2010), and ISPIRLI *et al*. (2017). However, our study yielded no evidence of β-hemolytic activity among the isolated strains, with all tested isolates exhibiting α-hemolytic characteristics exclusively.

Given the unsuitability of β-hemolytic strains for food applications and their undesirability as starter cultures in food fermentations, our findings underscore the importance of excluding such strains from food production processes. Nonetheless, it is crucial to recognize that non-hemolytic *Enterococcus* species isolated from food may still raise safety concerns when considered for use as starter cultures, emphasizing the need for comprehensive safety assessments and risk management strategies (DE VUYST *et al*., 2003).

Enterococci are increasingly recognized for their ability to develop resistance to a wide range of antibiotics, posing significant challenges in clinical and food safety settings (OGIER AND SERROR, 2008). Antibiotic-resistant strains of *Enterococcus* have been frequently isolated from raw foods, suggesting that food and water may serve as potential vectors for the transmission of resistant strains to the human intestinal flora (GIRAFFA, 2002; WITTE, 2000). Our study revealed a notable prevalence of antibiotic resistance among enterococci, with the highest resistance recorded against vancomycin (22.54%), consistent with previous reports indicating high resistance rates to vancomycin among enterococci isolated from cheeses made with raw ewe's milk (SALAMANDANE *et al*., 2023). Additionally, our findings are in line with those of JAHANSEPAS *et al*. (2022), who reported a higher prevalence of antibiotic resistance among *Ent. faecalis* strains compared to *Ent. faecium* (Table 4), further underscoring the importance of surveillance and management of antibiotic resistance in enterococci. Interestingly, our study identified streptomycin and gentamicin as effective antibiotics against the tested enterococcal isolates, with all strains exhibiting sensitivity to streptomycin and a significant portion of *Ent. faecium* and *Ent. hirae* isolates demonstrating sensitivity to gentamicin. Notably, only one *Ent. faecium* isolate (C21-5) displayed resistance to ampicillin, highlighting the variable resistance profiles among enterococcal strains and the importance of ongoing monitoring and surveillance efforts to mitigate the spread of antibiotic resistance in foodborne pathogens.

Antagonistic potential of selected Enterococcus spp. isolates

In this study, we assessed the potential of isolated *Enterococcus* to inhibit the growth of indicator strains using the agar-well diffusion method. The indicator strains included *E. coli* ATCC 25922, *P. mirabilis* ATCC 12453, *S. aureus* ATCC 25923, *E. coli* G14, and *K. pneumoniae*. Table 5 summarizes the antibiotic sensitivity of the tested indicator strains, while Table 6 lists the isolates that exhibited antagonistic potential, with inhibition zone diameters exceeding 6 mm for at least one indicator strain.

Table 5. Antibiotic susceptibility of indicator strains (GRUJOVIĆ *et al*., 2024)

Antibiotic	***S. aureus* ATCC 25923**	***P. mirabilis* ATCC 12453**	***E. coli* ATCC 25922**	***E. coli* G14**	***K. pneumoniae***
Amoxicillin	24 (S)	24 (S)	16 (S)	20	/
Chloramphenicol	26 (S)	45 (S)	31 (S)	24	22
Tetracycline	28 (S)	10 (R)	22 (S)	20	20

Zone of growth inhibition given in mm (millimeter); S-sensitive; R-resistant.

The inhibition zone diameters of *Ent. faecium* isolates against *S. aureus* ATCC 25923 ranged from 6 to 8 mm, against *P. mirabilis* ATCC 12453 and *E. coli* G14 from 6 to 10 mm, and against *E. coli* ATCC 25922 from 3 to 10 mm. Only *Ent. faecium* C14-2 and *Ent. faecium* C28-2 isolates showed limited activity against *K. pneumoniae*.

For *Ent. faecalis* isolates, inhibition zone diameters against *S. aureus* ATCC 25923 ranged from 6 to 10 mm, against *P. mirabilis* ATCC 12453 from 4 to 8 mm, against *E. coli* G14 from 8 to 10 mm, and against *E. coli* ATCC 25922 from 4 to 8 mm. Activity against *K. pneumoniae* was observed only for *Ent. faecalis* C0-9 isolate (6 mm).

GRUJOVIĆ *et al*. (2024), showed that tested indicator strains exhibited sensitivity to all antibiotics tested (Table 5), except for *P. mirabilis* ATCC 12453. Through comparative analysis with antibiotic inhibition zone diameters against various indicator strains in our investigation, it could be concluded that the tested *Enterococcus* isolates showed limited activity.

Among the eight *Ent. faecalis* strains isolated from raw goat milk (Table 1), only two (M-4 and M-6) demonstrated antagonistic potential against the tested indicator strains (Table 6). Additionally, ten isolates exhibiting antagonistic potential were obtained from goat cheese.

The utilization of *Enterococcus* isolated from raw milk and cheese as antimicrobial agents presents both opportunities and challenges across various applications. *Enterococcus* species are recognized for their capacity to produce antimicrobial compounds, such as bacteriocins, effectively inhibiting the growth of pathogenic bacteria and spoilage microorganisms. For instance, the natural isolate from cheese, *Ent. faecium* RZS C5, has demonstrated robust activity against *L. monocytogenes* (LEROY *et al*., 2003). Studies by ÖZMEN TOĞAY *et al*. (2016) further corroborated the antimicrobial efficacy of *Enterococcus* spp. isolated from traditional Turkish cheese against various pathogenic strains. MURUZOVIĆ *et al*. (2018) and GRUJOVIĆ *et al*. (2019) reported that *Enterococcus* isolates derived from raw cow's cheese exhibited moderate inhibitory activity against indicator strains including *E. coli* ATCC 25922, *P.mirabilis* ATCC 12453, *K. oxytoca* KGPMF1, *K. ornithinolytica* KGPMF8, and *Aeromonas hydrophila*, with inhibition zone diameters ranging from 10-14 mm. Conversely, isolates from our study demonstrated reduced activity, with inhibition zone diameters ranging from 4-10 mm. CAVICCHIOLI *et al*. (2019) highlighted the bacteriocin-producing ability of *Ent. hirae* isolated from Brazilian artisanal cheese. However, our findings revealed no antagonistic effect of *Ent. hirae* isolates against the tested indicator strains. These results suggest that the observed inhibitory effects may vary among different *Enterococcus* isolates and are likely strain-specific.

While *Enterococcus*-derived antimicrobial compounds offer significant benefits in food preservation by extending shelf life through bacterial inhibition (LEROY *et al*., 2003; HASSANZADAZAR *et al*., 2014), concerns exist regarding their use. One major consideration is the potential for horizontal gene transfer, which could facilitate the transmission of antibiotic resistance genes to pathogenic bacteria, posing risks in food safety and exacerbating antibiotic resistance concerns in the food chain (PANDOVA *et al*., 2024). Additionally, the safety assessment of *Enterococcus* strains as antimicrobial agents is paramount to mitigate potential health risks, particularly given their association with opportunistic infections in immunocompromised individuals (SEMEDO-LEMSADDEK *et al*., 2009; GRUJOVIĆ *et al*., 2022). Rigorous testing protocols are essential to evaluate the safety of *Enterococcus* strains intended for food production. Moreover, the possibility of off-flavors or off-odors arising from the metabolic activities of *Enterococcus* strains underscores the need for comprehensive risk assessment and careful consideration of their use in food applications (ÖZTÜRK *et al*., 2023).

Table 6. Antagonistic potential of isolated LAB

Species	Isolate	Indicator strains									
		S. aureus ATCC 25923		*P. mirabilis* ATCC 12453		*E. coli* ATCC 25922		*E. coli* G14		*K. pneumoniae*	
		ZI	A	ZI	A	ZI	A	ZI	A	ZI	A
Ent. faecium	C14-2	6	T	/	/	4	C	8	T	4	T
Ent. faecium	C21-4	6	T	10	T	/	/	10	T	/	/
Ent. faecium	C28-1	8	T	6	C	4	T	8	T	/	/
Ent. faecium	C28-2	6	T	8	T	6	T	6	T	6	T
Ent. faecium	C28-4	8	T	/	/	3	T	8	T	/	/
Ent. faecalis	M-4	10	T	8	C	/	/	8	T	/	/
Ent. faecalis	M-6	8	T	/	/	4	T	8	T	/	/
Ent. faecalis	C0-7	/	/	/	/	/	/	8	T	/	/
Ent. faecalis	C0-9	8	T	6	T	8	C	8	T	6	T
Ent. faecalis	C28-3	10	T	4	T	8	C	8	T	/	/
Ent. faecalis	C28-7	6	T	/	/	8	C	10	T	/	/
Ent. faecalis	C28-12	8	T	6	T	6	T	10	T	/	/

ZI*, zone of growth inhibition given in mm (millimeter); A, zone appearance (C, clear zone of inhibition; T, turbid zone of inhibition;
/, no zone of inhibition)

CONCLUSION

The usage of enterococci in the food industry is limited because they can carry different virulence factors and genes resistant to various antibiotics which are often used for disease treatment. Despite that facts, the properties of enterococci are strain-dependent and some strains could be safe. The usage of *Enterococcus* isolated from raw goat milk and goat cheese as antimicrobial agents holds promise for enhancing food safety and quality, particularly in dairy products. However, careful consideration must be given to safety, regulatory compliance, and potential technological limitations when incorporating *Enterococcus* strains into food production processes. Further research is needed to fully understand the benefits and risks associated with using *Enterococcus* as antimicrobial agents and to develop effective strategies for their application in food production.

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