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Probiotic Characteristics of Enterocin-Producing Enterococci Isolated from Traditional Turkish Cheeses

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ABSTRACT: This study aimed to evaluate the probiotic properties of the bacteriocin-producing strains *Enterococcus faecium* DP8.3, *E. faecium* DP9.3 and *E. mundtii* DP35.1. All strains were found to survive at pH 3.0 and maintained their viability in the presence of 0.4% phenol and 100 ppm lysozyme. In simulated gastric juice adjusted to pH 3.0, the viable cell counts of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were 8.96±0.01, 8.37±0.00, and 8.63±0.21 $\log_{10}$ CFU/mL, respectively. The bacteriocin-producing strains were capable of surviving at bile salt concentrations of 0.3%, 0.5%, and 1%. Additionally, these strains were able to grow in the presence of taurocholic acid, glycocholic acid, taurodeoxycholic acid sodium salts, and glycodeoxycholic acid sodium salts. The strains exhibited high hydrophobicity, autoaggregation, and coaggregation abilities. According to the results, these bacteriocin-producing strains possess probiotic properties, suggesting their potential use as protective probiotic cultures in food production. Further studies, including in vivo studies and food trials, are required to assess the potential of these strains as protective probiotic cultures in the food industry.

Keyword: *Enterococcus*; bacteriocin; probiotic properties; cheese.

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INTRODUCTION

Probiotics are living microorganisms that provide health benefits to the host when consumed in sufficient quantities (FAO/WHO, 2002). These positive health effects include lowering intestinal pH, producing enzymes and vitamins, balancing intestinal microflora, reducing serum cholesterol, stimulating the immune system, decreasing food allergen reactions, relieving symptoms of lactose intolerance, and improving calcium absorption and the effectiveness of antibiotic treatment (Kumar and Singh, 2009). For probiotic microorganisms to survive in foods and supplements, they must be resistant to the environmental conditions they encounter. Additionally, to remain alive and maintain their activity in the gastrointestinal tract (GIT), they must withstand saliva, gastric juice, bile salts and the competitive conditions of the intestine (Binda et al., 2020). It is also noted that probiotics ensure the safety and extend the shelf life of food products by producing various metabolites, such as bacteriocins, organic acids, hydrogen peroxide, and diacetyl, as well as by competing with pathogens (Hossain et al., 2017; Khaneghah et al., 2019).

Lactic acid bacteria (LAB) are a group of beneficial microorganisms that can exhibit probiotic properties and prevent the development of pathogenic microorganisms through their various antimicrobial agents (Daba et al., 2021). Among LAB, certain species of *Lactobacillus* are known to exhibit well-documented probiotic properties. Moreover, species belonging to the LAB genera *Lactococcus*, *Streptococcus*, *Pediococcus*, and *Enterococcus* have also been reported to exhibit probiotic potential (Anjana and Tiwari, 2022). Enterococci are LAB commonly found in the gastrointestinal tract of humans and animals. They are also found in various foods and the environment due to their ability to adapt to and survive in extreme conditions, such as high temperature, pH, and salt concentrations (Graham et al., 2020). In order to produce traditional fermented foods, enterococci play a critical role. They contribute to the development of the taste, aroma, and textural properties of fermented milk products through various mechanisms, such as acid production, lipolytic and proteolytic activity (Bagci et al., 2019; Graham et al., 2020). Studies on traditional cheeses produced from raw milk in Mediterranean countries have shown that enterococci contribute to the characteristic taste and aroma of the products through their proteolytic and lipolytic activities, as well as citrate metabolism (Foulquié Moreno et al., 2006; Terzić-Vidojević et al., 2021). Since the degradation of casein through proteolytic and peptidolytic activities is crucial for the development of cheese texture and organoleptic properties, enterococci contribute positively to cheese production due to their proteolytic activity. Also enterococci are utilized in ripened dairy products due to their ability to exhibit esterolytic and lipolytic activities (Terzić-Vidojević et al., 2021). Additionally, due to their technological properties, enterococci are also evaluated for their use as probiotics because they are naturally present in the gastrointestinal systems of humans and animals and contribute to digestive health. Several studies have shown that probiotic enterococci offer a range of health benefits, such as aiding in the treatment of diarrhea, viral infections, and diseases caused by foodborne pathogens (Lau and Chamberlain, 2016). Furthermore, they exhibit anti-mutagenic and anti-carcinogenic properties, enhance intestinal mucosal barrier function (Ahl et al., 2016), stimulate the immune system (Sheikhi et al., 2016), and aid in cholesterol assimilation (Kobyliak et al., 2016). Studies have shown that certain strains of *Enterococcus faecium* and *E. faecalis* are utilized as commercial probiotics (Pajarillo et al., 2015; Zommiti et al., 2022). Along with their technological and probiotic properties, most enterococcal isolates also produce bacteriocins known as enterocins, which exhibit inhibitory activity against spoilage bacteria and foodborne pathogens (Graham et al., 2020). Owing to their ability to produce bacteriocins, enterococci have been proposed for use as biopreservatives in dairy products (Toplu and Özden Tuncer, 2023), as well as in meat and poultry products (Chakchouk-Mtibaa et al., 2017; Orihuel et al., 2018).

Some strains of enterococci can cause endocarditis, bacteremia, intra-abdominal inflammation, urinary tract infections, and hospital-acquired infections (Kristich et al., 2014). They are also considered opportunistic pathogens due to their increasing antibiotic resistance and virulence factors (Graham et al., 2020). Unlike other probiotic microorganisms, enterococci are not recognized as Generally Recognized as Safe (GRAS) in the USA and lack Qualified Presumption of Safety (QPS) status in the European Union (EU), despite their potential use as adjunct, protective, or probiotic cultures. This is due to their capacity to acquire genetic determinants of virulence and antibiotic resistance, as well as the opportunistic pathogenicity of certain strains (Dapkevicius et al., 2021). However *E. faecium* SF68 and *E. faecalis*

DSM 16431 (under the trade name Symbioflor 1) are among the most commonly utilized probiotic strains within the genus *Enterococcus*. *E. faecium SF68 has been reported to be effective in reducing the incidence of antibiotic-associated diarrhea, and its supplementation in dry dog food has been shown to significantly enhance immune function in puppies (*Wunderlich et al., 1989; Benyacoub et al., 2003*).* Symbioflor 1 is recommended for the treatment of acute and recurrent sinusitis or bronchitis (Krawczyk et al., 2021).

Therefore, it is crucial to focus on safety evaluations when studying the probiotic and technological characteristics of enterococcal strains. This study examined the probiotic properties of bacteriocin-producing *E. faecium* DP8.3, *E. faecium* DP9.3, and *E. mundtii* DP35.1 which were isolated from different homemade traditional Turkish cheeses. Technological and safety evaluations of these strains were conducted previously, and the strains were found to be susceptible to clinically important antibiotics and did not harbor antibiotic resistance or virulence factor genes (Akpınar Kankaya, 2024).

MATERIAL AND METHODS

Bacterial strains and cultivation conditions

The bacteriocin-producing *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were previously isolated from traditional Turkish cheeses (Akpınar Kankaya, 2024). During the experiments, these strains were cultured in De Man Rogosa and Sharpe (MRS) broth (Biokar Diagnostics, BK070HA, Beauvais, France) at 37°C for 24 hours.

Survival at low pH

The survivability of the bacteriocin-producing strains at low pH was determined using the method described by Conway et al. (1987). After growing in MRS broth for 24 hours, the strains were centrifuged at 3000 x *g* for 10 minutes (Sigma 2-16P, Germany) and subsequently washed with phosphate-buffered saline (PBS). Then, 0.1 mL of the cell suspension was transferred into 2 mL of PBS at pH 1.0, 3.0, and 5.0. At 0, 1, 2, 3, and 4 hours of incubation, cell counts were conducted. The experiments were performed using a PBS solution at pH 7.2 as a control.

Tolerance to 0.4% phenol

Phenol resistance was assessed in MRS broth medium containing 0.4% phenol (w/v, Riedel-de Haën, Germany). Active cultures of *Enterococcus* strains were transferred (2%, v/v) into MRS broth with or without phenol and incubated at 37 °C for 24 hours. Cell numbers were determined at 0 and 24 hours of incubation (Teply et al., 1984).

Determination of lysozyme resistance

The impact of lysozyme on the growth of *Enterococcus* strains was tested in MRS broth containing 100 mg/L lysozyme (Sigma-Aldrich, USA). Strains were grown in MRS broth with or without lysozyme at 37°C for 24 hours and, cell count were enumerated at 0 and 24 hours of incubation (Brennan et al., 1986).

Tolerance to simulated gastric juice

The resistance of *Enterococcus* strains to simulated gastric juice was tested in simulated gastric juice at pH 2.0 and pH 3.0. *Enterococcus* strains grown at 37°C for 24 hours were centrifuged at 6000 x *g* for 20 minutes. The cell pellets were washed with 50 mM K_2HPO_4 (pH 6.5) buffer and then resuspended in this buffer. Next, 1 mL of cell suspension was centrifuged at 12000 x *g* for 5 minutes, dissolved in 10 mL of simulated gastric juice (0.3% pepsin and 0.5% NaCl, adjusted to pH 2.0 and pH 3.0), and incubated at 37°C. The number of viable cells was assessed after 0 and 3 hours of incubation (Vinderola and Reinheimer, 2003).

Resistance to bile salt

To determine the bile salt resistance of *Enterococcus* strains, overnight cultures were inoculated at 1% (v/v) into MRS broth containing 0.3%, 0.5% or 1% (w/v) bile salt and incubated at 37 C. Cell counts in MRS broth without bile salt were used as a control in the experiments, and cell counts were enumerated at 0 and 24 hours of incubation (Gilliland and Walker, 1990).

Bile salt deconjugation

Elliker broth was used to cultivate *Enterococcus* strains for 18 hours at 37°C. Ten µL of the overnight culture were then transferred to Elliker agar that contained 0.5% (w/v) sodium salts of glycocholic acid, glycodeoxycholic acid, taurocholic acid, or taurodeoxycholic acid. A positive result was indicated by the presence of a precipitation zone of deconjugated bile salts around the colonies after 72 hours of incubation at 37°C. Additionally, cell growth was evaluated as positive, weak, or negative in the presence of sodium salts (Öztürk et al., 2024).

Hydrophobicity

The hydrophobicity of *Enterococcus* strains was measured using the method outlined by Vinderola

and Reinheimer (2003). Overnight cultures were centrifuged at 12000 x *g* for 5 minutes, and pellets were washed with 50 mM K_2HPO_4 buffer. Cell suspensions were prepared in the same buffer. The absorbance of the cell suspensions was adjusted to approximately 1.0 at 560 nm using a UV/VIS spectrophotometer (Soif UV-5100, Turkey). After that, 3 mL of the cell suspension was vortexed for two minutes with 0.6 mL of n-hexadecane added. For phase separation, the tubes were kept at 37°C, and the absorbance of the aqueous phase was measured at 560 nm. The formula for calculating the hydrophobicity percentage of the isolates is provided below.

Hydrophobicity (%) = $[(A_0\text{-}A) / A_0] \times 100$

In the formula, A_0 and A denote the absorbance values before and after treatment with n-hexadecane, respectively.

Autoaggregation and coaggregation

To determine autoaggregation abilities of the strains, cell pellets were washed, resuspended in PBS buffer, and the absorbance was adjusted to 0.3 ± 0.005 at 660 nm. Following a 60-minute incubation period at 37°C, the cell suspensions precipitated, and the absorbance of the supernatant was measured. Autoaggregation ability was assessed using the following formula, in which A_0 denotes the initial optical density of the strains, while A_{60} represents the optical density measured after 60 minutes of incubation at room temperature (Basson et al., 2008).

Autoaggregation (%) = $[(A_0\text{-}A_{60}) / A_0] \times 100$

Coaggregation abilities of bacteriocin-producing *Enterococcus* strains with *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25923 and *Salmonella* Typhimurium ATCC 14028 were evaluated using the method described by Basson et al. (2008). The absorbance of cell pellets was adjusted to 0.3 at 660 nm after resuspension in PBS. Subsequently, equal volumes of the pathogen culture suspensions and 0.5 mL of the strains' cell suspensions were mixed and incubated for 60 minutes at 37 °C. Following incubation, the absorbance of the supernatant was measured at 660 nm. Coaggregation ability was determined using the following formula, where A_0 corresponds to the initial optical density of the mixed strains, and A_{60} corresponds to the optical density recorded after 60 minutes of incubation at room temperature.

Coaggregation (%) = $[(A_0\text{-}A_{60}) / A_0] \times 100$

RESULTS

Survival at low pH

Based on the survival results at low pH, it was determined that all strains had cell counts ranging from 7.47 to 8.45 $\log_{10}$ CFU/mL at the beginning of incubation at pH 1.0, but all strains decreased to an undetectable level (<1 $\log_{10}$ CFU/mL) within the first hour at pH 1.0 (Table 1). All strains were found to survive during the 4-hour incubation period at pH 3.0 and pH 5.0. After 4 hours of incubation at pH 3.0, the survival rates of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were determined to be 94.67%, 73.98%, and 72.1%, respectively. The survival rates of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 at pH 5.0 were found to be 100.73%, 92.64%, and 99.89%, respectively.

Tolerance to 0.4% phenol

Upon examining the survival characteristics of bacteriocin-producing *Enterococcus* strains in the presence of 0.4% phenol, it was found that all strains preserved their viability throughout the 24-hour incubation period (Table 1). *E. faecium* DP9.3 exhibited growth in the presence of 0.4% phenol, while *E. faecium* DP8.3 and *E. mundtii* DP35.1 preserved their viability.

Determination of lysozyme resistance

It was determined that all *Enterococcus* strains grew in the presence of 100 ppm lysozyme (Table 1). After 24 hours of incubation in MRS broth containing 100 ppm lysozyme, the cell numbers of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 increased by 22.18%, 9.64%, and 24.76%, respectively.

Tolerance to simulated gastric juice

In this study, all *Enterococcus* strains lost their viability after 3 hours of incubation in simulated gastric juice at pH 2.0 (Table 1). However, it was determined that these strains maintained their viability after 3 hours of incubation in simulated gastric juice at pH 3.0.

Resistance to bile salt

It was found that bacteriocin-producing *Enterococcus* strains maintained their viability at 0.3%, 0.5%, and 1% bile salt concentrations during the 24-hour incubation period (Table 1). It was observed that *E. mundtii* DP35.1 grew at 0.3% bile salt, while it maintained viability at 0.5% and 1% bile salt concentrations. The cell numbers of *E. faecium* DP8.3 and DP9.3 strains increased at all bile salt concentrations.

Table 1. Various probiotic properties of *Enterococcus* strains

Treatment	pH / concentration	Time	Cell number (log_{10} CFU/mL)		
			E. faecium **DP8.3**	*E. faecium* **DP9.3**	*E. mundtii* **DP35.1**
Resistance to low pH	pH 1.0	0	8.02±0.25	7.47±0.07	8.45±0.04
		1	< 1	< 1	< 1
		2	< 1	< 1	< 1
		3	< 1	< 1	< 1
		4	< 1	< 1	< 1
	pH 3.0	0	8.26±0.05	8.11±0.09	8.54±0.03
		1	8.55±0.01	7.86±0.18	8.73±0.04
		2	8.32±0.14	7.41±0.27	8.55±0.35
		3	8.24±0.25	7.00±0.00	6.57±0.14
		4	7.82±0.03	6.00±0.00	6.15±0.04
	pH 5.0	0	8,26±0.06	8.42±0.08	8.79±0.38
		1	8.26±0,06	8.02±0.31	8.52±0.17
		2	8.56±0.00	7.41±0.16	8.40±0.39
		3	8.42±0.08	7.79±0.44	8.54±0.03
		4	8.32±0.28	7.80±0.54	8.78±0.03
	pH 7.2	0	8.32±0.28	8.30±0.0	8.46±0.15
		1	8.16±0.28	8.18±0.66	8.73±0.05
		2	8.62±0.25	8.39±0.08	8.10±0.17
		3	8.40±0.17	8.21±0.13	8.36±0.10
		4	8.26±0.24	8.34±0.03	8.80±0.08
Phenol	0.4 %	0	7.26±0.06	7.30±0.00	7.33±0.05
		24	4.42±0.08	7.69±0.08	6.89±0.12
Lysozyme	100 ppm	0	7.17±0.07	7.26±0.06	7.15±0.21
		24	8.76±0.09	7.96±0.13	8.92±0.10
Simulated gastric juice	pH 2.0	0	9.10±0.05	8.59±0.23	8.69±0.08
		3	<1	<1	<1
	pH 3.0	0	8.92±0.02	8.52±0.00	8.72±0.08
		3	8.96±0.01	8.37±0.00	8.63±0.21
Bile salt resistance	0.3%	0	6.92±0.02	6.80±0.03	6.84±0.09
		24	8.54±0.04	7.88±0.03	7.79±0.17
	0.5%	0	6.82±0.03	6.86±0.18	6.80±0.19
		24	8.42±0.12	7.48±0.00	4.84±0.03
	1%	0	6.68±0.17	6.90±0.14	6.62±0.35
		24	8.35±0.03	7.70±0.00	4.06±0.09

Bile salt deconjugation

It was observed that all *Enterococcus* strains exhibited weak growth in the presence of glycocholic acid and glycodeoxycholic acid sodium salts, but they grew well in the presence of taurocholic acid and taurodeoxycholic acid sodium salts. No precipitation zones were observed on any of the Petri dishes containing bile salts. It was determined that the strains did not deconjugate bile salts, but continued to grow in their presence.

Hydrophobicity

In this study, the hydrophobicity rates of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were 93.78±1.71%, 52.06±1.39%, and 83.03±3.73%, respectively (Figure 1).

Autoaggregation and coaggregation

E. faecium DP8.3, DP9.3, and DP35.1 exhibited high autoaggregation abilities of 65.64±2.24%, 77.56±4.67% and 49.42±0.82%, respectively (Figure 1).

The coaggregation rates of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 with *S. aureus* ATCC 43300 were 74.13±4.85%, 84.79±5.47%, and 59.27±1.32%, respectively. With *E. coli* ATCC 25923, the coaggregation rates of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were 67.80±4.03%, 67.49±1.16%, and 50.80±1.33%, respectively. The coaggregation rates with *S.* Typhimurium ATCC 14028 for *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 were 69.12±0.36%, 70.45±2.97%, and 43.44±0.03%, respectively. Finally, the coaggregation rates with *L. monocytogenes* ATCC 7644 were 53.66±0.12%, 58.19±0.20%, and 41.70±0.43% for *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1, respectively (Figure 2). All strains showed the highest coaggregation ability with *S. aureus* ATCC 43300.

DISCUSSION

In recent years, the positive effects of probiotic supplements and foods on health have gained increasing acceptance among both researchers and consumers. This trend has contributed to the accelerated research on new probiotic strains. Among probiotic cultures, enterococci, in particular, stand out from other LAB. Although enterococci are predominantly found in the GIT of humans and animals, they are also capable of surviving in food matrices due to their ability to grow under extreme conditions such as varying temperatures, pH levels, and high salt concentrations. The ability of enterococci with probiotic properties to produce bacteriocins further enhances their significance in the food industry. In selecting a strain as a potential probiotic, factors such as its ability to survive under stress conditions-like the acidity of the gastrointestinal tract and the presence of bile salts-along with its capacity to produce various antimicrobial substances are considered (FAO/WHO, 2002).

Probiotic microorganisms should be capable of colonizing the GIT and withstand stress conditions for an extended period. A key criterion for selecting a probiotic strain is its ability to survive at low pH levels, similar to gastric juice (pH 2.0-3.0), for up to 3 hours (Daba et al., 2021; Yıldırım and Özden Tuncer, 2022). Since the pH of the stomach contents rises above 3.0 after food intake, it is considered sufficient for probiotic microorganisms to tolerate pH 3.0 for 3 hours in order to pass through the stomach (Shi et al., 2020). In this study, all strains were found to remain viable throughout the 4-hour incubation

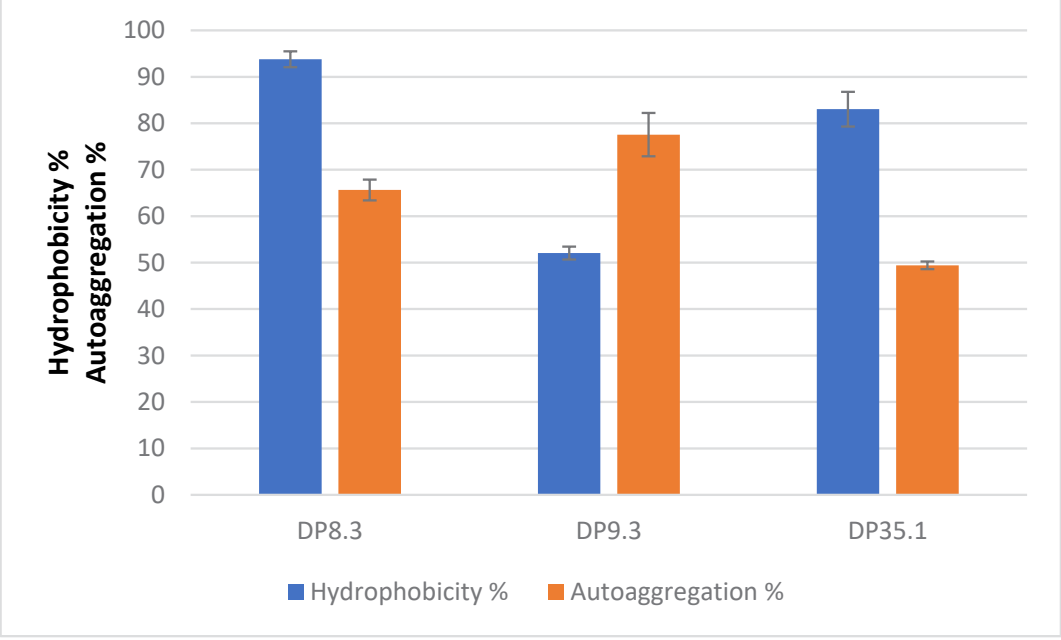

Figure 1. Hydrophobicity and autoaggregation abilities of *Enterococcus* strains.

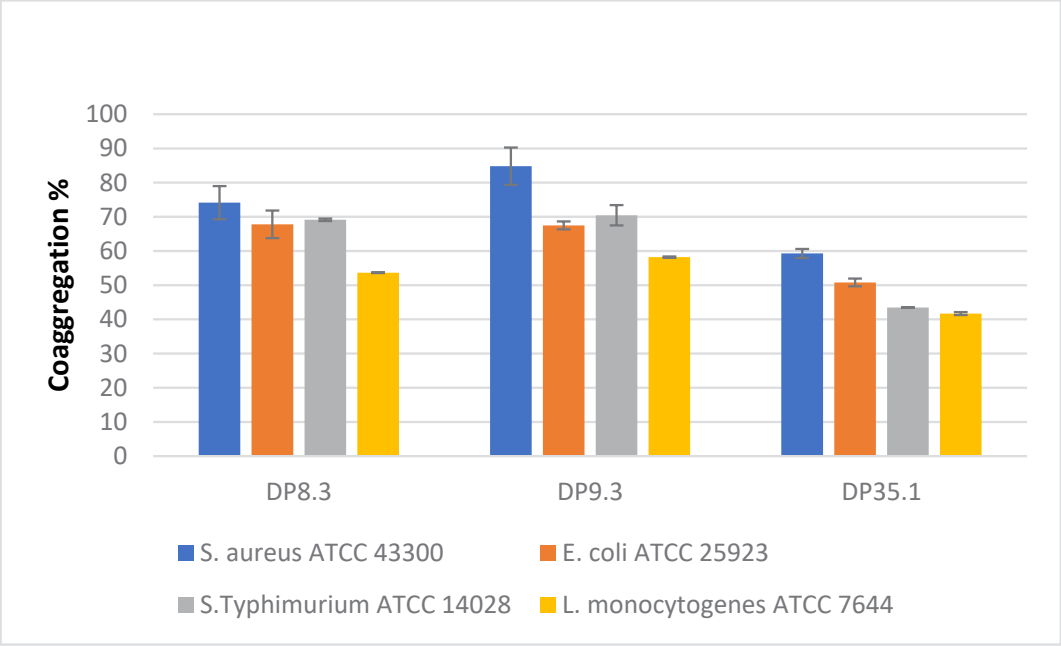

Figure 1. Coaggregation ability of *Enterococcus* strains..

period at both pH 3.0 and pH 5.0. These results were consistent with those reported by Nami et al. (2019), and Toplu and Özden Tuncer (2023). The number of viable cells was found to be at the recommended levels for probiotic bacteria (Binda et al., 2020).

Surviving in the upper GIT is a key factor in selecting probiotic strains that can provide beneficial effects on the digestive system (Bagci et al., 2019). In this study, *Enterococcus* strains were observed to maintain their viability after 3 hours of incubation in simulated gastric juice adjusted to pH 3.0. The *E. faecium* strains DP8.3 and DP9.3, along with the *E. mundtii* strain DP35.1, exhibited viability rates of 100.45%, 98.24%, and 98.97%, respectively, after 3 hours of incubation in simulated gastric juice at pH 3.0. Similarly, Bagci et al. (2019), Toplu and Özden Tuncer (2023), and Çetin and Aktaş (2024) reported that bacteriocin-producing enterococci strains exhibited high survival rates in simulated gastric juice adjusted to pH 3.0. Bacteria used for probiotic purposes are usually taken orally with food. Therefore, probiotic bacteria are expected to withstand the enzymes in the oral cavity and the digestive processes in the stomach and intestines. Although the minimum time required for probiotics to cross this barrier is 90 minutes, probiotic bacteria are expected to withstand acidic conditions for up to 3 hours, as other digestive processes take longer (Chou and Weimer, 1999). The pH is above 4.0 for 73% of the food intake period and above 5.0 for 45% of that period, due to the buffering capacity of the foods (Dressman et al., 1990). Since all *Enterococcus* strains were found to maintain their viability at pH 3.0, it is likely that they can survive the harsh conditions of the stomach and pass into the intestine at high rates.

Phenol, which can be produced in the intestine through the bacterial deamination of certain aromatic amino acids, possesses bacteriostatic properties. Therefore, resistance to phenol is a crucial trait for probiotic bacteria to sustain their viability in the GIT (Rajput et al., 2022). In this study, it was found that bacteriocin-producing *Enterococcus* strains maintained their viability during the 24-hour incubation period in the presence of 0.4% phenol. In line with the findings of this study, it was reported that bacteriocin-producing enterococci isolated from different foods exhibit phenol resistance (Toplu and Özden Tuncer 2023; Öztürk et al., 2024).

Lysozyme hydrolyzes the bacterial cell wall, resulting in cell death. Since lysozyme is naturally found in biological fluids such as saliva, the resistance of probiotic microorganisms to lysozyme may influence the colonization of these bacteria in the intestines (Yıldırım and Özden Tuncer, 2022). It was found that bacteriocin-producing *Enterococcus* strains maintained their viability after 24 hours in MRS broth containing 100 ppm lysozyme. Similarly,

several researchers have reported that enterococcal strains isolated from different foods are resistant to lysozyme (Ayyash et al., 2018; Toplu and Özden Tuncer, 2023; Öztürk et al., 2024). On the other hand, it has been noted that differences in the cell wall structure and layers of bacteria may lead to variations in their resistance to lysozyme (Sarkar et al., 2020).

Bile salt resistance is crucial for the colonization of probiotic strains, as the small intestine and colon contain relatively high concentrations of bile salts. Therefore, it is crucial for probiotic bacteria to be capable of growing in environments containing 0.15-0.3% bile salt (Ahmadova et al., 2013). In our study, it was found that *Enterococcus* strains maintained their viablety at bile salt concentrations of 0.3%, 0.5%, and 1% during the 24-hour incubation period. Similar to our results, Ahmadova et al. (2013) noticed that antimicrobial *E. faecium* AQ71, an isolate from Motal cheese, grew well in a medium containing 0.2% to 3% bile salt. Additionally, Nami et al. (2019) reported that the survival rate of *E. faecium* ES4 was 70.1%, while *E. faecium* ES27 had a survival rate of 68.3% at a bile salt concentration of 0.3%. Yerlikaya and Akbulut (2020) also reported that *E. faecium* and *E. durans* strains isolated from raw milk and traditional dairy products showed growth at a 1% bile salt concentration. Çetin and Aktaş (2024) reported that antilisterial *E. faecium* strains BH04, BH12, BH84, and BH99 can tolerate bile salt concentrations ranging from 0.3% to 1%. Öztürk et al. (2024) found that bacteriocin-producing *Enterococcus* strains survived at bile salt concentrations of 0.3%, 0.5% and 1%.

Strains that produce bile salt hydrolysis enzymes offer benefits such as bile salt detoxification, prolonged strain retention in the GIT, improved nutrient absorption, and protection against certain types of cancer (Nascimento et al., 2019). Additionally, probiotic bacteria in the intestine contribute to reducing serum cholesterol levels by deconjugating bile salts (Corzo and Gilliland, 1999). For probiotic bacteria to survive in the digestive system and exhibit positive health effects, the bile salt deconjugation capability of probiotic strains is an important feature that should be assessed. In this study, the *Enterococcus* strains were unable to deconjugate bile salts, but they continued to grow in their presence. Similarly, Yerlikaya and Akbulut (2020) reported that some enterococci isolated from various dairy products and raw milk showed growth in the presence of taurocholic acid (TC), glycocholic acid (GC), glycodeoxycholic acid (GDC), and taurodeoxycholic acid (TDC). On the other hand, some bacteriocin-producing *Enterococcus* strains have been reported to exhibit bile salt hydrolysis in different studies (Bagci et al., 2019; Çetin and Aktaş, 2024; Öztürk et al., 2024).

Hydrophobicity is a key physicochemical property that influences the adhesion of probiotics to intestinal epithelial cells. It can vary based on changes in the physiological state of cells, differences in the expression of surface-associated proteins across strains, and variations between species (Olajugbagbe et al., 2020). High hydrophobicity rates in probiotics increase their ability to adhere to epithelial surfaces and, consequently, enhance their positive health effects (Nami et al., 2019). It is stated that the cell surface hydrophobicity for probiotic strains should be at least 40% (Son et al., 2018). This study found that the hydrophobicity rates of the *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 strains were 93.78±1.71%, 52.06±1.39%, and 83.03±3.73%, respectively. Bagci et al. (2019) reported that the hydrophobicity of bacteriocin-producing *Enterococcus* strains ranged from 35 to 56% for xylene and 37 to 47% for n-octane. Toplu and Özden Tuncer (2023) found that the hydrophobicity value of the bacteriocin-producing *E. faecium* BT29.11 strain was 44.35±0.71% for xylene. In contrast to the findings of this study, some studies have reported that bacteriocin-producing enterococci strains isolated from different dairy products exhibit lower hydrophobicity values (Favaro et al., 2014; Santos et al. 2015).

Autoaggregation is the process where bacteria of the same species clump together, whereas coaggregation involves the clumping of bacteria from different species. It is recognized that autoaggregation is linked to the adhesion to epithelial cells (Nami et al., 2019). The autoaggregation values of *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 strains were found to be 65.64±2.24%, 77.56±4.67%, and 49.42±0.82%, respectively, after 24 hours. Similar to our results, Nami et al. (2019) found that the autoaggregation abilities of bacteriocin-producing *Enterococcus* strains obtained from artisanal dairy products ranged from 24.7±2.3 to 81.2±2.6%. Toplu and Özden Tuncer (2023) reported an autoaggregation value of 56.89±2.47% for the bacteriocin-producing *E. faecium* BT29.11, which was isolated from Turkish White cheese. In contrast to our results, Ayyash et al. (2018) reported the autoaggregation values of antimicrobial *Enterococcus* strains isolated

from camel milk ranged from 5.7±2.25 to 33.1±0.25 at the and of 24 hours.

Coaggregation serves as a protective barrier against the colonization of pathogenic microorganisms (Nami et al. 2019). This is an important characteristic of probiotics to prevent the development of pathogens in the intestinal tract, thereby reducing the incidence of pathogen-related diseases (Ayyash et al., 2018). Some researchers reported that coaggregation varies according to strain, species and incubation time (Ayyash et al., 2018; Nami et al., 2019). Similar to our results, Nami et al. (2019) found that the coaggregation rates of antimicrobial-active *Enterococcus* strains ranged from 2.2-12.7% with *S. aureus*, 2.8-19.9% with *E. coli*, and 2.5-18.7% with *L. monocytogenes*. Toplu and Özden Tuncer (2023) noticed that the coaggregation percentage of *E. faecium* BT29.11 with *L. monocytogenes* ATCC 7644 was 43.95±1.78%. Öztürk et al. (2024) observed that the coaggregation percentages of 13 enterocin-producing *Enterococcus* strains ranged from 24.78±0.30-37.86±0.50% with *S. aureus*, 36.00±1.89-46.47±0.96% with *E. coli*, 32.85±0.73- 43.66±1.26% with *S.* Typhimurium, and 38.04±1.14-45.35±0.34% with *L. monocytogenes* after 24 hours of incubation.

CONCLUSION

This study examined the probiotic properties of bacteriocin-producing *Enterococcus* strains that were previously isolated from traditional Turkish cheeses. These strains were found to maintain their viability under certain stress conditions. All three *Enterococcus* strains survived at pH 3.0 and were resistant to 0.4% phenol and 100 ppm lysozyme. Furthermore, following 3 hours of incubation in simulated gastric juice at pH 3.0, it was found that all *Enterococcus* strains maintained their viability. The strains were also able to grow with different bile salts, such as taurocholic acid, glycocholic acid, taurodeoxycholic acid sodium salts, and glycodeoxycholic acid sodium salts, and they remained viable even after being exposed to 1% bile salt. Additionally, the strains exhibited high hydrophobicity, autoaggregation, and coaggregation abilities. The data from this study showed that the bacteriocin-producing strains *E. faecium* DP8.3, DP9.3, and *E. mundtii* DP35.1 exhibited good probiotic properties. However, to be recognized as potential probiotic cultures applicable in the food industry, these strains require more comprehensive studies, including both in vivo experiments and food trials, to assess their properties under real conditions.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

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