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EFFECT OF DIFFERENT CULTURE MEDIA, INITIAL pH, INCUBATION TEMPERATURE, AND CARBON SOURCES ON GROWTH AND BACTERIOCIN PRODUCTION OF *ENTEROCOCCUS MUNDTII* YB6.30

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ABSTRACT
This study investigated the effects of various factors on the production of mundticin-KS in the *Enterococcus mundtii* YB6.30 strain, previously isolated from Sucuk, a dry-fermented sausage. The examined factors included the culture medium (de Man, Rogosa, and Sharpe broth; Brain Heart Infusion broth; M17 broth; Luria-Bertani broth; and Trypticase Soy Broth), initial medium pH (4.5, 5.5, 6.2, 7.4, and 8.5), incubation temperature (25°C, 30°C, 37°C, and 40°C), carbon source (glucose, fructose, lactose, or sucrose), and sucrose concentration (1%, 2%, 3%, 4%, 5%, 7.5%, and 10%). A one-factor-at-a-time (OFAT) approach was employed to determine the factors influencing bacteriocin production. Statistical analysis revealed that optimal mundticin-KS production by *E. mundtii* YB6.30 was achieved after 6 and 8 hours of incubation at 30°C in modified MRS broth medium, adjusted to an initial pH of 6.2 with the addition of 1% (w/v) sucrose. The bacteriocin activity under these conditions was measured at 1495.62±29.93 AU/mL at the 6th hour and 1567.21±26.27 AU/mL at the 8th hour (P>0.05).
Keywords: *Enterococcus mundtii*, mundticin-KS, bacteriocin production, culture medium, incubation temperature, carbon source

FARKLI KÜLTÜR BESİYERİ, BAŞLANGIÇ PH'SI, İNKÜBASYON SICAKLIĞI VE KARBON KARNAKLARININ *ENTEROCOCCUS MUNDTII* YB6.30'UN GELİŞİMİ VE BAKTERİYOSİN ÜRETİMİ ÜZERİNE ETKİSİ

ÖZ
Bu çalışma, daha önce kuru fermente sucuklardan izole edilen *Enterococcus mundtii* YB6.30 suşunda mundtisin-KS üretimi üzerine çeşitli faktörlerin etkilerini incelemiştir. İncelenen faktörler arasında kültür ortamı (de Man, Rogosa ve Sharpe broth; Brain Heart Infusion broth; M17; Luria-Bertani broth; ve Tryptic Soy broth), başlangıç pH'ı (4.5, 5.5, 6.2, 7.4 ve 8.5), inkübasyon sıcaklığı (25°C, 30°C, 37°C ve 40°C), karbon kaynağı (glikoz, fruktoz, laktoz veya sakkaroz) ve sakkaroz

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konsantrasyonu (%1, %2, %3, %4, %5, %7.5 ve %10) yer almaktadır. Bakteriyosin üretimini etkileyen faktörleri belirlemek için one-factor-at-a-time (OFAT) yaklaşımı kullanılmıştır. İstatistiksel analiz, *E. mundtii* YB6.30 tarafından optimal mundtisin-KS üretiminin, 1% (w/v) sakkaroz eklenmiş başlangıç pH'ı 6.2'ye ayarlanmış modifiye MRS broth besiyerinde 30°C'de 6 ve 8 saatlik inkübasyon sonrası elde edildiğini ortaya koymuştur. Bu koşullar altında bakteriyosin aktivitesi 6. saatte 1495.62±29.93 AU/mL ve 8. saatte 1567.21±26.27 AU/mL olarak ölçülmüştür (P>0.05).
Anahtar kelimeler: *Enterococcus mundtii*, mundtisin-KS, bacteriyosin üretimi, kültür besiyeri, inkübasyon sıcaklığı, karbon kaynağı

INTRODUCTION

Lactic acid bacteria (LAB) play a pivotal role in the development of the distinct flavor, aroma, scent, and texture of fermented foods (Laranjo et al., 2019; de Souza et al., 2023). Moreover, LAB are commonly employed as protective starter cultures in fermented foods due to their production of lactic acid, hydrogen peroxide, and bacteriocins, which possess antibacterial properties (Garneau et al., 2002; Gök Charyyev et al., 2019; de Souza et al., 2023). The use of bacteriocin-producing strains for food preservation is increasingly widespread, driven by growing consumer demand for foods preserved with natural preservatives and minimal processing, as opposed to those containing synthetic additives (Oliveira et al., 2018; Ren et al., 2021; Kasimin et al., 2022; Karnwal and Malik, 2024; Bisht et al., 2025). *Enterococcus*, a genus of LAB, is adapted to extreme environmental conditions, including wide pH ranges, elevated temperatures, and high salt concentrations (Álvarez-Cisneros and Ponce-Alquicira, 2019; Sakoui et al., 2024). These bacteria are naturally present in the gastrointestinal tracts of humans and animals, as well as in soil, plants, surface waters, feeds, and a variety of raw and fermented foods, such as milk, red meat, cheese, sausage, seafood, and poultry (Schelegueda et al., 2015; Hanchi et al., 2018; Akpınar Kankaya and Tuncer, 2020; Graham et al., 2020; Sakoui et al., 2024). Enterococci are also utilized as natural starter cultures that enhance the sensory characteristics and extend the shelf life of fermented foods such as cheeses and sausages. They contribute to the formation of flavor, odor, aroma, and texture through acidification, citrate degradation, and the production of volatile aromatic compounds (Graham et al., 2020). The bacteriocins produced by enterococci, called enterocins, exhibit strong antilisterial activity (Franz et al., 2007; Gök Charyyev et al., 2019; Graham et al., 2020; Kasimin et al., 2022; Öztürk et al., 2023). Among the enterocins, the most extensively studied strains are *E. faecium* and *E. faecalis* (Almeida-Santos et al., 2021), although there have been reports indicating that *E. mundtii* also produces bacteriocins (Kawamoto et al., 2002; Altınkaynak and Tuncer, 2020; Öztürk et al., 2023). Bacteriocins can be employed in the food industry as additives, incorporated into food packaging and coating materials, and integrated into starter culture formulations using producer strains (Ahmad et al., 2017; Bisht et al., 2025). Given their potential applications and the benefits they offer in terms of food safety, large-scale production of bacteriocins is essential for commercial and economic viability (Reuben and Torres, 2024). Consequently, investigating optimal fermentation conditions to maximize the growth and bacteriocin production of bacteriocin-producing strains is critical for achieving cost-effective production (Jawan et al., 2020). The efficiency of bacteriocin production in LAB is influenced by several factors. Cultural conditions, such as the type of culture medium, initial pH, incubation temperature and time, and carbon and nitrogen sources, have significant effects on bacterial growth and bacteriocin production, with these factors varying across different strains (Todorov et al. 2012; Abbasiliasi et al., 2017; Oliveira et al., 2024).

In this study, we examined the effects of various commercial culture media, initial pH levels, incubation temperatures, and carbon sources on the growth and bacteriocin production of the *E. mundtii* YB6.30 strain. The objective was to identify the optimal conditions for bacteriocin production using a one-factor-at-a-time (OFAT) method.

MATERIAL AND METHODS

Bacteria and growth conditions

In this study, the mundticin KS-producing *E. mundtii* YB6.30 strain, which was isolated from Sucuk -a dry fermented Turkish sausage (Altınkaynak and Tuncer, 2020- and obtained from the culture collection of the Süleyman Demirel University Food Engineering Department Bacterial Genetics Laboratory, was used. The *E. mundtii* YB6.30 stock culture, stored at -32°C, was introduced into de Man, Rogosa, and Sharpe (MRS) broth (Biokar Diagnostics, BK070HA, Beauvais, France) at a 1% (v/v) concentration for activation and incubated at 37°C for 18 h. Before use in experiments, the activated culture was subcultured twice in MRS broth. The working culture was stored at +4°C, while the stock culture was preserved at −32°C with 20% (v/v) sterile glycerol.

Control of bacteriocin activity

The activity of the bacteriocin produced by the *E. mundtii* YB6.30 was evaluated using the agar-spot method (Todorov and Dicks, 2009) and the agar-well diffusion method (Jawan et al., 2020). To obtain the cell-free supernatant (CFS), an overnight culture of *E. mundtii* YB6.30 was transferred into 10 mL sterile centrifuge tubes (Thermo Scientific, Oak Ridge Centrifuge Tube 3114-0010, United States) and centrifuged at 10.000 x *g* for 5 minutes at +4°C (Sigma 2-16KL, rotor no. 12141, Germany). Following centrifugation, the supernatant was transferred to a beaker glass, and its pH was adjusted to 6.0 using 1 N NaOH. The neutralized culture supernatant was then sterilized by filtration through a 0.45 µm pore-diameter membrane filter (Sartorius Stedim Biotech, Minisart® 16555-K, Germany). Next, 100 µL of *E. faecium* ATCC 51559 (vancomycin-resistant) was inoculated into 5 mL of MRS soft agar (0.5% agar), vortex-mixed, and spread on MRS agar plates to serve as the indicator bacterium. Bacteriocin activity was assessed using the agar-well diffusion and agar-spot methods. For the agar-well diffusion assay, a 6 mm-diameter probe was used to create a well in an MRS agar plate, which was then filled with 100 µL of neutralized CFS. In the agar-spot method, 10 µL of neutralized CFS was deposited onto an empty spot on the same MRS agar plate. The plates were incubated at 37°C for 18 h. Following incubation, bacteriocin activity of the *E. mundtii* YB6.30 was evaluated by measuring the zones of inhibition surrounding the well and the CFS application site.

Factors affecting cell growth and bacteriocin production

A one-factor-at-a-time (OFAT) method was employed to identify the variables affecting bacteriocin production. The most influential factors were selected for subsequent experiments based on their contribution to maximizing bacteriocin yield (Jawan et al., 2020).

Culture medium

The effects of various commercial media on cell growth and bacteriocin production were examined using MRS broth (Biokar, France), M17 broth (Biokar, K012HA, France), Luria Bertani broth (LB, Sigma-Aldrich, 28713, St. Louis, MO, USA), Brain Heart Infusion broth (BHI, Neogen Culture Media, NCM0016A, England), and Tryptone Soy Broth (TSB, Acumedia LabM, LAB004, England). *E. mundtii* YB6.30 was cultured overnight in MRS broth at 37°C and subsequently inoculated into 50 mL of MRS, M17, LB, BHI, and TSB broths at a 2% (v/v) concentration (Todorov and Dicks, 2009).

Initial pH of culture medium

In experiments conducted to determine the effect of the initial pH of the culture medium on cell growth and bacteriocin production, the initial pH values of MRS broth were adjusted to 4.5, 5.5, 6.2, 7.4, and 8.5 using 0.5 N HCl or NaOH (Yang et al., 2018). *E. mundtii* YB6.30 was then inoculated into 50 mL of MRS broth at a 2% (v/v) ratio and cultured at 37°C for 24 h (Todorov and Dicks, 2009).

Cultuvation temperature

To determine the effect of different incubation temperatures on cell growth and bacteriocin production, the *E. mundtii* YB6.30 was inoculated at a rate of 2% (v/v) into 50 mL of MRS broth with a pH adjusted to 6.2. The medium was then

incubated for 24 h at 25°C, 30°C, 37°C, and 40°C (Malheiros et al., 2015).

Carbone source

To investigate the impact of various carbon sources on cell growth and bacteriocin production, *E. mundtii* YB6.30 was cultured in modified MRS broths, which were prepared by adding 2% (w/v) glucose, fructose, lactose, or sucrose to basal MRS broth (peptone 20 g, yeast extract 5 g, Tween 80 1.08 g, dipotassium phosphate 2 g, sodium acetate 5 g, ammonium citrate 2 g, magnesium sulfate 0.2 g, manganese sulfate 0.05 g), with the pH adjusted to 6.2. *E. mundtii* YB6.30 was then inoculated into 50 mL of modified MRS broth at a 2% (v/v) inoculum concentration, and the media were incubated at 30°C for 24 h.

Sucrose concentration

To assess the effect of varying sucrose concentrations on cell growth and bacteriocin production in *E. mundtii* YB6.30, sucrose was incorporated into basal MRS broth at concentrations of 1%, 2%, 3%, 4%, 5%, 7.5%, and 10%, with the initial pH adjusted to 6.2. The YB6.30 strain was inoculated into the modified MRS broth at a 2% (v/v) inoculum, and the media were incubated at 30°C for 24 h.

Determination of pH, optical density and bacteriocin activity

During the incubation, samples were collected from the cultures at the 0th, 6th, 8th, 10th and 24th h to measure pH, optical density, and bacteriocin activity. Each experiment was performed in duplicate. The pH of *E. mundtii* YB6.30 cultures was measured using a WTW pH 3110 model pH meter (Germany). The optical density was measured with a Soif UV5100 UV/VIS spectrophotometer (Türkiye) at a wavelength of 600 nm, using cuvettes with a 1 cm light path. Bacteriocin activity was determined in AU/mL, using Petri dishes prepared according to Todorov and Dicks (2009), as described above. *E. faecium* ATCC 51559 was used as the indicator bacterium. A well was created in the Petri dish using a 6 mm-diameter probe, and 100 µL of the neutralized CFS, prepared as described above, was transferred with a pipette. The Petri plate was stored in the refrigerator at 4°C for two h, then incubated at 37°C for 18 hours. After the incubation, the activity zone formed around the well was measured with calipers, and bacteriocin activity was calculated using the formula below. A_z represents the clear zone area (mm^2) around the well, A_W is the well area (mm^2), and V is the volume of the sample (mL) (Jawan et al., 2020).

Bacteriocin activity (AU/mL): $(A_z - A_w)/V$ (1)

Statistical analysis

The data were analyzed using factorial designs with the repeated measures analysis of variance (ANOVA). Repeated measurements were conducted at different levels of the time factor, with each subgroup containing two observations. Following the analysis of variance, the Tukey test, a method for multiple comparisons, was employed to determine the differences between the factor level averages (Mendeş, 2019).

RESULTS AND DISCUSSION

Control of bacteriocin activity

The bacteriocin production capacity of *E. mundtii* YB6.30 was re-evaluated to assess whether any loss in bacteriocin production occurred during its storage in the culture collection. Altınkaynak and Tuncer (2020) reported that the sterile toothpick method revealed the *E. mundtii* YB6.30 strain produced a 19-mm zone of inhibition against *E. faecium* ATCC 51559. In the present study, the *E. mundtii* YB6.30 demonstrated efficacy against *E. faecium* ATCC 51559 using both the agar spot and well diffusion methods (Figure 1).

The genes responsible for bacteriocin production are located on chromosomal DNA, plasmids, or conjugative transposons (Fguira et al., 2014; Miljkovic et al., 2018). Accordingly, this study primarily examined the bacteriocin activity of *E. mundtii* YB6.30, which was reactivated from a stock culture stored at -20°C. Previous research has demonstrated that bacteriocins AS-48 (Martínez-Bueno et al., 1990) and enterocin 416K1 (Sabia et al., 2002), produced by *Enterococcus* strains, were plasmid-encoded, with

this trait being lost in mutant strains that underwent plasmid loss. Additionally, Kawamoto et al. (2002) reported that the genetic determinants of mundticin-KS were encoded on the 50 kb pML1 plasmid in *E. mundtii* NFRI 7393. Similarly, Feng et al. (2009) found that mundticin L production was plasmid-encoded in *E. mundtii* CUGF08. After evaluating the antibacterial activity of *E. mundtii* YB6.30, this study further investigated the effects of various commercial media, initial pH values, incubation temperatures, and different carbon sources on cell growth and bacteriocin activity.

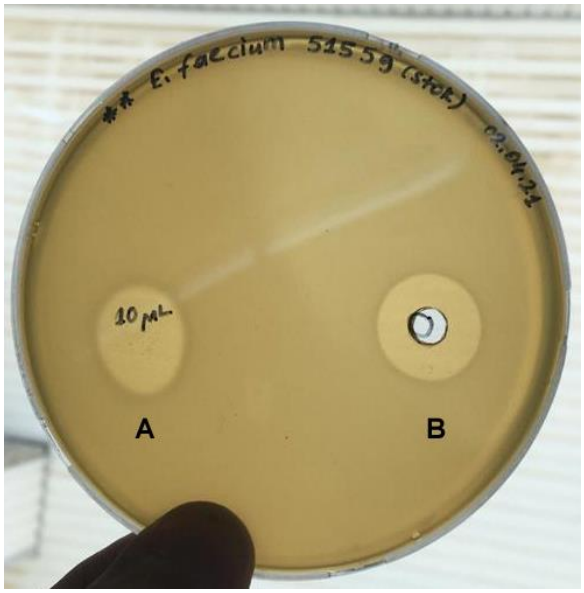

Figure 1. Antibacterial activity of *E. mundtii* YB6.30 against the indicator bacterium *E. faecium* ATCC 51559 (vancomycin-resistant). A: agar-spot method, B: agar-well diffusion method.

Effect of culture medium

The increasing importance of bacteriocins in the food industry has created a need for their production to be more cost-effective and yield higher quantities (Olivera et al., 2004; Garsa et al., 2014). Studies addressing this need primarily investigate the effect of culture media on bacteriocin production. The *E. mundtii* YB6.30 strain was observed to grow in all of the media used in the experiment and exhibited bacteriocin production (Table 1). At the end of the 24-hour incubation period, the lowest pH value was recorded in MRS broth. A statistically significant difference ($P<0.05$) was observed in the pH values across all media during the incubation period. The highest optical density values were recorded at the 6th and 8th hours of incubation in BHI broth, measuring 2.08±0.006 and 2.06±0.008, respectively ($P<0.05$). However, no statistical difference was observed between the optical densities in BHI and MRS broth at the 10th hour of incubation, and in MRS, M17, and BHI broth media at the 24th hour of incubation ($P>0.05$). As expected, the effect of medium type on bacteriocin activity at hour 0 was not statistically significant ($P>0.05$). After 6 hours of incubation, MRS broth was found to be more effective for bacteriocin production (1322.96±46.67 AU/mL) ($P<0.05$) compared to the other media, followed by BHI broth (876.26±46.67 AU/mL). Bacteriocin activity in M17, TSB, and LB media did not differ significantly ($P>0.05$). Throughout all incubation periods, the highest bacteriocin activity was detected in MRS broth ($P<0.05$). The peak bacteriocin activity (1739.60±14.09 AU/mL) was observed at the 24th hour of incubation. Based on these results, MRS medium was determined to be the most suitable for the production of mundticin-KS by the *E. mundtii* YB6.30.

Table 1. pH, optical density, and bacteriocin activity values of *E. mundtii* YB6.30 culture measured at 0, 6, 8, 10, and 24 hours of incubation in MRS, M17, TSB, BHI, and LB broth media.

Incubation time (hour)	Media*	Culture pH	Optical density	Bacteriocin activity (AU/mL)
0.	MRS	$5.94\pm0.009^{Ad**}$	0.04 ± 0.005^{Cb}	332.41 ± 26.99^{Da}
	M17	6.44 ± 0.009^{Ac}	0.10 ± 0.005^{Da}	291.44 ± 26.99^{Ca}
	TSB	6.84 ± 0.009^{Aa}	0.09 ± 0.005^{Ba}	291.27 ± 26.99^{Ca}
	BHI	6.85 ± 0.009^{Aa}	0.09 ± 0.005^{Ba}	326.01 ± 26.99^{Ca}
	LB	6.55 ± 0.009^{Ab}	0.10 ± 0.005^{Ba}	343.81 ± 26.99^{Ca}
6.	MRS	4.88 ± 0.014^{Be}	1.82 ± 0.006^{Bb}	1322.96 ± 46.67^{Ca}
	M17	5.89 ± 0.014^{Ba}	1.48 ± 0.006^{Cc}	643.12 ± 46.67^{Bc}
	TSB	5.28 ± 0.014^{Bc}	1.84 ± 0.006^{Ab}	737.66 ± 46.67^{ABc}
	BHI	5.06 ± 0.014^{Bd}	2.08 ± 0.006^{Aa}	876.26 ± 46.67^{Bb}
	LB	5.55 ± 0.014^{Bb}	0.83 ± 0.006^{Ad}	633.73 ± 46.67^{Bc}
8.	MRS	4.62 ± 0.010^{Cd}	2.00 ± 0.008^{Ab}	1591.24 ± 22.82^{Ba}
	M17	5.49 ± 0.010^{Ca}	1.84 ± 0.008^{Bc}	895.41 ± 22.82^{Ac}
	TSB	5.27 ± 0.010^{Bb}	1.85 ± 0.008^{Ac}	829.12 ± 22.82^{Ac}
	BHI	5.05 ± 0.010^{Bc}	2.06 ± 0.008^{Aa}	1044.36 ± 22.82^{Ab}
	LB	5.46 ± 0.010^{Ba}	0.86 ± 0.008^{Ad}	829.12 ± 22.82^{Ac}
10.	MRS	4.47 ± 0.009^{Ce}	2.02 ± 0.008^{Aa}	1664.87 ± 38.40^{Ba}
	M17	5.22 ± 0.009^{Dc}	1.97 ± 0.008^{ABb}	811.14 ± 38.40^{Abc}
	TSB	5.26 ± 0.009^{Bb}	1.87 ± 0.008^{Ac}	746.73 ± 38.40^{ABcd}
	BHI	5.05 ± 0.009^{Bd}	2.06 ± 0.008^{Aa}	885.87 ± 38.40^{Bb}
	LB	5.41 ± 0.009^{Ba}	0.86 ± 0.008^{Ad}	642.01 ± 38.40^{Bd}
24.	MRS	4.04 ± 0.007^{De}	2.03 ± 0.015^{Aa}	1739.60 ± 14.09^{Aa}
	M17	5.04 ± 0.007^{Ed}	2.01 ± 0.015^{Aa}	658.65 ± 14.09^{Bc}
	TSB	5.31 ± 0.007^{Bb}	1.82 ± 0.015^{Ab}	650.06 ± 14.09^{Bc}
	BHI	5.09 ± 0.007^{Bc}	2.03 ± 0.015^{Aa}	934.19 ± 14.09^{ABb}
	LB	5.48 ± 0.007^{Ba}	0.91 ± 0.015^{Ac}	658.81 ± 14.09^{Bc}

*MRS: de Man, Rogosa, and Sharpe broth; M17; M17 broth; TSB: Tryptone soy broth; BHI: Brain heart infusion broth; LB: Luria Bertani broth.

** Capital letters denote distinctions between time periods within each medium, while lowercase letters represent differences between media within each time period.

Consistent with previous research on other bacteriocin-producing *Enterococcus* strains, MRS broth has been identified as the most efficient medium for bacteriocin production compared to other commercially available media (Zendo et al., 2005; De Kwaadsteniet et al., 2005; Yang et al., 2018). Zendo et al. (2005) evaluated various media, including MRS broth, APT medium, M17 broth, BHI broth, Elliker broth, and tryptic soy broth, for bacteriocin production by *E. mundtii* QU2. Their results showed that MRS broth yielded the highest bacteriocin production (102.400 AU/mL), while APT medium supported the greatest cell growth. Similarly, De Kwaadsteniet et al. (2005) reported that *E. mundtii* ST15 exhibited the highest bacteriocin activity (51.200 AU/mL) when grown in MRS broth, compared to BHI broth, M17 broth, soymilk (10% w/v, soy flour), and molasses (8% and 10% w/v). Yang et al. (2018) also identified MRS broth as the optimal medium for bacteriocin production by *E. faecium* JR-1 compared to BHI broth. Furthermore, they found that MRS broth was also the optimal medium for the bacteriocin activity in other LAB species, including *Lactobacillus curvatus* Arla-10, *Lactobacillus paracasei* subsp. *paracasei* JFR-5, and *Streptococcus thermophilus* TSB-8. In another study, Parlindungan et al. (2021) reported that *Lactiplantibacillus plantarum* B21 exhibited higher cell growth and bacteriocin production in MRS broth than in M17 broth. Oliveira et al. (2024) found that *Pediococcus pentosaceus* ST65ACC produced higher levels of bacteriocin when grown in MRS broth compared to BHI broth, meat broth, and reconstituted skim milk. Conversely, other studies have reported that media other than

MRS broth support maximum bacteriocin production in LAB (Sidek et al., 2018; Jawan et al., 2020; Valledor et al., 2022). Sidek et al. (2018) identified M17 as the optimal medium for the growth and bacteriocin activity of *Pediococcus acidilactici* kp10. Jawan et al. (2020) reported that *Lactococcus lactis* Gh1 exhibited the highest bacteriocin production activity in BHI broth. Valledor et al. (2022) found that among two enterocin-producing *E. faecium* isolates from kimchi, the ST20Kc strain exhibited the highest bacteriocin activity (25.600 AU/mL) when cultured BL and M17 (containing 0.5% glucose) broths, while the ST41Kc strain showed the highest activity in BL broth.

Effect of initial pH of culture medium

It was determined that *E. mundtii* YB6.30 exhibited growth and bacteriocin activity at all initial pH values, although at varying levels (Table 2). The highest optical densities of the culture, observed at the 6th, 8th, and 10th hours of incubation, were recorded in MRS broth with an initial pH of 6.2 ($P<0.05$). However, by the end of the 24th hour of incubation, no statistically significant difference was found between the optical densities in MRS broth with initial pH values of 6.2 and 8.5 ($P>0.05$). The highest bacteriocin activity, measured at 1790.31±24.56 AU/mL, occurred at the 6th hour of incubation in MRS broth with an initial pH of 6.2 ($P<0.05$). Bacteriocin activity decreased during the later hours of incubation. Conversely, the highest bacteriocin activity (1358.09±51.00 AU/mL) was recorded at the 10th hour of incubation in MRS broth with an initial pH of 8.5. By the end of the 24th hour of incubation, the highest bacteriocin activity values were found to be 1777.63±27.76 AU/mL and 1777.87±27.76 AU/mL in MRS broth adjusted to pH 5.5 and 8.5, respectively.

Table 2. pH, optical density, and bacteriocin activity values of *E. mundtii* YB6.30 culture measured at 0, 6, 8, 10, and 24 hours of incubation in MRS broth, with pH levels adjusted to 4.5, 5.5, 6.2, 7.4, and 8.5

Incubation time (hour)	Initial pH of the medium	Culture pH	Optical density	Bacteriocin activity (AU/mL)
0.	4.5	$4.58\pm0.012^{Ae*}$	0.08 ± 0.006^{Ca}	118.73 ± 12.06^{Bc}
	5.5	5.37 ± 0.012^{Ad}	0.10 ± 0.006^{Da}	346.38 ± 12.06^{Ca}
	6.2	5.86 ± 0.012^{Ac}	0.07 ± 0.006^{Ba}	201.16 ± 12.06^{Cb}
	7.4	6.44 ± 0.012^{Ab}	0.08 ± 0.006^{Ba}	396.43 ± 12.06^{Ca}
	8.5	6.96 ± 0.012^{Aa}	0.05 ± 0.006^{Ba}	367.46 ± 12.06^{Da}
6.	4.5	4.56 ± 0.017^{Ab}	0.16 ± 0.017^{Cd}	130.04 ± 24.56^{Bd}
	5.5	4.98 ± 0.017^{Ba}	1.25 ± 0.017^{Cc}	1391.02 ± 24.56^{Bc}
	6.2	4.75 ± 0.017^{Bb}	1.93 ± 0.017^{Aa}	1790.31 ± 24.56^{Aa}
	7.4	4.69 ± 0.017^{Bb}	1.85 ± 0.017^{Ab}	1579.19 ± 24.56^{Ab}
	8.5	5.02 ± 0.017^{Ba}	1.84 ± 0.017^{Ab}	1425.29 ± 24.56^{BCc}
8.	4.5	4.53 ± 0.027^{Aa}	0.21 ± 0.017^{Cd}	107.58 ± 27.45^{Bd}
	5.5	4.69 ± 0.027^{Ba}	1.72 ± 0.017^{Bc}	1367.94 ± 27.45^{Bc}
	6.2	4.56 ± 0.027^{Ba}	2.03 ± 0.017^{Aa}	1689.67 ± 27.45^{Aa}
	7.4	4.55 ± 0.027^{Ba}	1.93 ± 0.017^{Ab}	1531.14 ± 27.45^{Ab}
	8.5	4.62 ± 0.027^{Ca}	1.94 ± 0.017^{Ab}	1495.62 ± 27.45^{Bb}
10.	4.5	4.53 ± 0.026^{Aa}	0.29 ± 0.009^{Bd}	213.72 ± 51.00^{Bc}
	5.5	4.55 ± 0.026^{Ba}	1.90 ± 0.009^{ABc}	1311.46 ± 51.00^{Bab}
	6.2	4.43 ± 0.026^{Ba}	2.05 ± 0.009^{Aa}	1300.59 ± 51.00^{Bab}
	7.4	4.44 ± 0.026^{Ba}	1.94 ± 0.009^{Abc}	1245.05 ± 51.00^{Bb}
	8.5	4.48 ± 0.026^{Ca}	1.97 ± 0.009^{Ab}	1358.09 ± 51.00^{Ca}
24.	4.5	4.35 ± 0.104^{Aa}	0.90 ± 0.007^{Ad}	418.60 ± 27.76^{Ad}
	5.5	4.11 ± 0.104^{Ba}	1.95 ± 0.007^{Ab}	1777.63 ± 27.76^{Aa}
	6.2	4.10 ± 0.104^{Bb}	2.05 ± 0.007^{Aa}	1289.17 ± 27.76^{Bc}
	7.4	4.31 ± 0.104^{Bab}	1.86 ± 0.007^{Ac}	1483.65 ± 27.76^{Ab}
	8.5	4.12 ± 0.104^{Cb}	2.03 ± 0.007^{Aa}	1777.87 ± 27.76^{Aa}

*Capital letters denote differences between times at each pH level, while lowercase letters indicate differences between pH values at each time point.

Similar to our findings, Yang et al. (2018) reported that an initial pH of 6.2 in MRS broth was optimal for bacteriocin production in several LAB strains. Additionally, Cladera-Olivera et al. (2004) examined the effect of initial pH on bacteriocin activity in *Bacillus licheniformis* and found that activity was highest between pH 6.5 and 7.5. Conversely, Jawan et al. (2020) observed that *Lactococcus lactis* Gh1 exhibited maximum bacteriocin activity in BHI broth at initial pH values of 7.4 and 8.5. Furthermore, bacteriocin activity declined when the initial pH dropped below 6.0. These studies collectively highlight the crucial role of initial pH in cell growth and bacteriocin production, while also indicating that the optimal pH varies by strain (Cladera-Olivera et al., 2004; Yang et al., 2018; Jawan et al., 2020).

Effect of incubation temperature

The results of pH, optical density, and bacteriocin activity measurements, taken at 0, 6, 8, 10 and 24 hours of incubation at different temperatures, are presented in Table 3. It was found that the differences in pH values measured at all incubation temperatures from the 6th hour of incubation was statistically significant ($P<0.05$). The YB6.30 strain exhibited the greatest increase in cell density during the 6th hour of incubation at 37°C, with an optical density measurement of 1.92±0.018. However, at the 6th hour of incubation, no statistically significant difference was detected in bacteriocin activity between cultures incubated at 30°C, 37°C, and 40°C ($P>0.05$). At the 8th hour of incubation, the highest cell density was measured in the culture incubated at 37°C ($P<0.05$), while the maximum bacteriocin activity (1752.28±30.49 AU/mL) was observed in the culture incubated at 30°C ($P<0.05$). When evaluating bacteriocin activity in the culture incubated at 30°C, no statistical difference was found between the values measured at 6 and 8 hours of incubation ($P>0.05$).

Table 3. pH, optical density, and bacteriocin activity values of *E. mundtii* YB6.30 culture measured at 0, 6, 8, 10, and 24 hours of incubation in MRS broth (initial pH 6.2) at 25°C, 30°C, 37°C, and 40°C.

Incubation time (hour)	Incubation temperature (°C)	Culture pH	Optical density	Bacteriocin activity (AU/mL)
0.	25	5.94±0.011^{Aa*}	0.06±0.002^{Ea}	271.37±15.89^{Dab}
	30	5.94±0.011^{Aa}	0.08±0.002^{Ca}	339.28±15.89^{Cab}
	37	5.96±0.011^{Aa}	0.07±0.002^{Ba}	245.31±15.89^{Cb}
	40	5.94±0.011^{Aa}	0.07±0.002^{Ba}	389.24±15.89^{Ca}
6.	25	5.80±0.008^{Ba}	0.42±0.018^{Dc}	1169.34±44.00^{Cb}
	30	5.29±0.008^{Bb}	1.44±0.018^{Bb}	1690.46±44.00^{Aa}
	37	4.74±0.008^{Bc}	1.92±0.018^{Aa}	1714.48±44.00^{Aa}
	40	4.62±0.008^{Bd}	1.87±0.018^{Aa}	1603.36±44.00^{Aa}
8.	25	5.64±0.012^{Ba}	0.88±0.017^{Cc}	1356.68±30.49^{Bc}
	30	4.92±0.012^{Cb}	1.88±0.017^{Ab}	1752.28±30.49^{Aa}
	37	4.54±0.012^{Cc}	1.99±0.017^{Aa}	1640.22±30.49^{Aab}
	40	4.46±0.012^{Cd}	1.93±0.017^{Ab}	1507.36±30.49^{Ab}
10.	25	5.36±0.013^{Ca}	1.41±0.019^{Bc}	1379.28±37.04^{Ba}
	30	4.72±0.013^{Db}	2.03±0.019^{Aa}	1437.18±37.04^{Ba}
	37	4.44±0.013^{Cc}	2.01±0.019^{Aa}	1413.99±37.04^{Ba}
	40	4.31±0.013^{Cd}	1.93±0.019^{Ab}	1223.38±37.04^{Bb}
24.	25	4.53±0.004^{Da}	2.09±0.010^{Aa}	1591.23±35.81^{Aa}
	30	4.27±0.004^{Eb}	2.08±0.010^{Aa}	1677.39±35.81^{Aa}
	37	4.09±0.004^{Dc}	2.01±0.010^{Aa}	1322.73±35.81^{Bb}
	40	3.99±0.004^{Dd}	1.96±0.010^{Ab}	1322.96±35.81^{Bb}

* Capital letters denote the differences between times at each temperature, while lowercase letters indicate the differences between temperatures at each time.

Studies conducted in previous years have demonstrated that incubation temperature significantly affects bacteriocin production and that optimal incubation conditions for cell growth and bacteriocin production may vary depending on the strain (Cladera-Olivera et al., 2004; De Kwaadsteniet et al., 2005; Bayram and Yıldırım, 2016; Mohd Rasid et al., 2023). Cladera-Olivera et al. (2004) found that *Bacillus licheniformis* produced bacteriocins most effectively at 26°C and 37°C. Similarly, De Kwaadsteniet et al. (2005) reported that *E. mundtii* ST15 exhibited the highest bacteriocin activity at 30°C compared to 37°C, a finding consistent with our study. Bayram and Yıldırım (2016) examined the effects of various incubation temperatures (25°C, 30°C, and 37°C) on bacteriocin production in the *E. faecium* BP strain and concluded that 30°C and 37°C were optimal. Likewise, Mohd Rasid et al. (2023) found that the cell growth and nisin activity of *Lactococcus lactis* ATCC 11454 were higher at 30°C than at 37°C.

Effect of using different carbon sources in culture medium

The results of the culture pH, optical density, and bacteriocin activity of the YB6.30 strain, cultured in modified MRS broth with different carbon sources, measured at 0, 6, 8, 10, and 24 hours of incubation, are presented in Table 4. Statistically significant differences ($P<0.05$) were observed between the culture pHs measured at the 6th and 8th hours of incubation. However, no statistically significant difference was found between the culture pHs measured in MRS broth containing sucrose and fructose at the 10th and 24th hours of incubation ($P>0.05$), while the culture pHs measured in MRS broth containing glucose or lactose were statistically significant ($P<0.05$). The lowest pH values were observed in *E. mundtii* YB6.30 cultures grown in MRS broth containing sucrose or fructose at the 24th hour of incubation. It was found that the highest cell density at each hour of the incubation occurred in samples cultured in MRS broth containing sucrose ($P<0.05$). Regarding bacteriocin activity, the highest activity (1507.36±21.94 AU/mL) was measured in the sample cultured in MRS broth containing sucrose at the 6th hour of incubation ($P<0.05$). At the 8th hour of incubation, the highest bacteriocin activity (1652.66±39.39 AU/mL) was observed in the sample cultured in MRS broth containing sucrose. However, at this time, no statistically significant difference was found between the bacteriocin activity of the sample cultured in MRS broth containing sucrose and that cultured in MRS broth containing glucose ($P>0.05$). At the 10th and 24th hours of incubation, no statistical difference was observed between the bacteriocin activities of all samples cultured in modified MRS broth ($P>0.05$). Based on these findings, sucrose was determined to be the optimal carbon source for inducing high bacteriocin activity in *E. mundtii* YB6.30 when cultivated in MRS broth.

Consistent with our findings, Saraiva et al. (2020) reported that sucrose was the most effective carbon source for bacteriocin production in *Lactococcus lactis*, a nisin Z producer isolated from Brazilian fermented sausage. Conversely, Todorov and Dicks (2009) examined the effects of various carbon sources on bacteriocin production in *E. mundtii* ST4SA and found that the highest bacteriocin activity (102.400 AU/mL) achived when fructose (20 g/L) was used as the carbon source in MRS broth. They also reported that when the same concentration of glucose was used, bacteriocin activity decreased to 51.200 AU/mL. Similarly, other studies have demonstrated that different carbon sources influence bacteriocin production across various LAB genera (Turgis et al., 2016; Jawan et al., 2020; Kuhan Sreedharan et al., 2021; Tareq and Luti, 2022). Turgis et al. (2016) found that the highest nisin production in *Lactococcus lactis* MM19 occurred in the presence of glucose and lactose, while the highest pediocin production in *Pediococcus acidilactici* MM33 was observed with galactose and fructose. Jawan et al. (2020) reported that fructose was the most effective carbon source for bacteriocin production in *L. lactis* Gh1. Kuhan Sreedharan et al. (2021) found that *Lactobacillus brevis* C23 exhibited the highest production of bacteriocin-like antibacterial substances when cultured with lactose. Tareq and Luti (2022) determined that glucose was the most suitable carbon source for enhancing both cell growth and bacteriocin production in *Lactobacillus crispatus* IS30.

Table 4. pH, optical density, and bacteriocin activity values of *E. mundtii* YB6.30 culture measured at 0, 6, 8, 10, and 24 hours of incubation at 30°C in MRS broth (initial pH 6.2) supplemented with glucose, lactose, sucrose, or fructose.

Incubation time (hour)	Carbone sources	Culture pH	Optical density	Bacteriocin activity (AU/mL)
0.	Glucose	5.69± 0.008^{Ac}	0.07± 0.002^{Cb}	298.06± 14.40^{Da}
	Lactose	5.90± 0.008^{Ab}	0.08± 0.002^{Cab}	339.28± 14.40^{Ea}
	Sucrose	6.12± 0.008^{Aa}	0.09± 0.002^{Ba}	291.43± 14.40^{Ea}
	Fructose	5.62± 0.008^{Ad}	0.08± 0.002^{C}	304.78± 14.40^{Ca}
6.	Glucose	5.43± 0.007^{Bc}	1.09± 0.005^{Bb}	1472.07± 21.94^{Bab}
	Lactose	5.83± 0.007^{Aa}	1.06± 0.005^{Bc}	1289.17± 21.94^{Bab}
	Sucrose	5.61± 0.007^{Bb}	1.40± 0.005^{Aa}	1507.36± 21.94^{Ba}
	Fructose	5.33± 0.007^{Bd}	0.94± 0.005^{Bd}	1234.10± 21.94^{Bb}
8.	Glucose	4.94± 0.008^{Cd}	1.67± 0.006^{ABb}	1615.73± 39.39^{Aa}
	Lactose	5.48± 0.008^{Ba}	1.42± 0.006^{ABd}	1333.91± 39.39^{Ab}
	Sucrose	4.95± 0.008^{Cb}	1.90± 0.006^{Aa}	1652.66± 39.39^{Aa}
	Fructose	4.87± 0.008^{Cc}	1.48± 0.006^{ABc}	1555.32± 39.39^{Aab}
10.	Glucose	4.74± 0.004^{Db}	1.82± 0.005^{ABb}	1158.70± 19.60^{Ca}
	Lactose	5.20± 0.004^{Ca}	1.65± 0.005^{ABd}	983.53± 19.60^{Da}
	Sucrose	4.70± 0.004^{Dc}	1.98± 0.005^{Aa}	1095.74± 19.60^{Da}
	Fructose	4.69± 0.004^{Dc}	1.70± 0.005^{Ac}	1148.14± 19.60^{Ba}
24.	Glucose	4.26± 0.003^{Eb}	1.97± 0.010^{Ab}	1158.70± 25.35^{Ca}
	Lactose	4.49± 0.003^{Da}	1.98± 0.010^{Aab}	1054.45± 25.35^{Ca}
	Sucrose	4.18± 0.003^{Ec}	2.00± 0.010^{Aa}	1223.38± 25.35^{Ca}
	Fructose	4.19± 0.003^{Ec}	1.83± 0.010^{Ac}	1148.14± 25.35^{Ba}

* Capital letters denote the differences between times at each carbone sources, while lowercase letters indicate the differences between carbon sources at each time.

Effect of adding different rates of sucrose in culture medium

The effects of adding different sucrose concentrations (1%, 2%, 3%, 4%, 5%, 7.5%, and 10%) to MRS broth on cell growth and bacteriocin production by *E. mundtii* YB6.30 are presented in Table 5. The pH values of cultures grown in MRS broth with varying sucrose concentrations differed significantly over incubation time (P<0.05); however, no statistically significant differences were observed at each measurement time (P>0.05). Similarly, the optical densities of cultures containing 1%, 2%, and 3% sucrose did not differ significantly at the 6th and 8th hours of incubation (P>0.05). Nevertheless, the highest bacteriocin activity was observed in the medium containing 1% sucrose during both incubation times (P<0.05). Specifically, bacteriocin activity was measured at 1495.62±29.93 AU/mL at the 6th hour and 1567.21±26.27 AU/mL at the 8th hour (P>0.05). Bacteriocin activity declined at later incubation times compared to the 6th and 8th hours. These findings indicate that a 1% sucrose concentration is optimal for maximizing bacteriocin activity in *E. mundtii* YB6.30.

Consistent with our findings, previous studies have reported greater bacteriocin activity with a low-rate carbon source than with a high-rate carbon source (Pattnaik et al., 2005; Todorov & Dicks, 2009; Valledor et al., 2022). Pattnaik et al. (2005) tested glucose at concentrations of 0.5%, 1%, 1.5%, and 2% as a carbon source for the production of lichenin, a bacteriocin produced by *Bacillus licheniformis* 26L-10/3RA, and found that higher carbon source concentrations negatively affected bacteriocin activity. Similarly, Todorov and Dicks (2009) reported that enterocin production in *E. mundtii* ST4SA peaked with the addition of 1.5% or 2% fructose to the culture medium. Valledor et al. (2022) also observed that enterocin-producing *E. faecium* ST20Kc and ST41Kc exhibited higher bacteriocin activity in MRS broth with 0.5% glucose than in MRS broth with 5% glucose.

Table 5. pH, optical density, and bacteriocin activity values of *E. mundtii* YB6.30 culture, measured at 0, 6, 8, 10, and 24 hours of incubation at 30°C in MRS broth (initial pH 6.2), supplemented with different concentrations of sucrose.

Incubation time (hour)	Sucrose concentration (%)	Culture pH	Optical density	Bacteriocin activity (AU/mL)
0.	1	$6.14\pm0.002^{Aa*}$	0.11 ± 0.008^{Ca}	226.12 ± 13.25^{Ca}
	2	6.19 ± 0.002^{Aa}	0.11 ± 0.008^{Ca}	232.52 ± 13.25^{Ca}
	3	6.19 ± 0.002^{Aa}	0.11 ± 0.008^{Ca}	264.90 ± 13.25^{Ca}
	4	6.18 ± 0.002^{Aa}	0.10 ± 0.008^{Ca}	271.37 ± 13.25^{Da}
	5	6.15 ± 0.002^{Aa}	0.08 ± 0.008^{Da}	170.89 ± 13.25^{Ca}
	7.5	6.11 ± 0.002^{Aa}	0.08 ± 0.008^{Ca}	176.98 ± 13.25^{Ca}
	10	6.13 ± 0.002^{Aa}	0.09 ± 0.008^{Da}	219.80 ± 13.25^{Da}
6.	1	5.54 ± 0.010^{Ba}	1.51 ± 0.013^{Ba}	1495.62 ± 29.93^{Aa}
	2	5.53 ± 0.010^{Ba}	1.53 ± 0.013^{Ba}	1413.79 ± 29.93^{Aab}
	3	5.55 ± 0.010^{Ba}	1.50 ± 0.013^{Ba}	1402.36 ± 29.93^{Aab}
	4	5.51 ± 0.010^{Ba}	1.44 ± 0.013^{Bb}	1311.62 ± 29.93^{ABb}
	5	5.79 ± 0.010^{Ba}	1.12 ± 0.013^{Cc}	1322.73 ± 29.93^{Bb}
	7.5	5.76 ± 0.010^{Ba}	1.04 ± 0.013^{Bd}	1334.07 ± 29.93^{Bb}
	10	5.80 ± 0.010^{Ba}	0.94 ± 0.013^{Ce}	1367.94 ± 29.93^{ABb}
8.	1	5.02 ± 0.008^{Ca}	1.90 ± 0.008^{Aa}	1567.21 ± 26.27^{Aa}
	2	5.02 ± 0.008^{Ca}	1.91 ± 0.008^{Aa}	1425.44 ± 26.27^{Abc}
	3	5.03 ± 0.008^{Ca}	1.88 ± 0.008^{Aa}	1345.18 ± 26.27^{Ac}
	4	4.99 ± 0.008^{Ca}	1.83 ± 0.008^{Ab}	1425.44 ± 26.27^{Abc}
	5	5.18 ± 0.008^{Ca}	1.73 ± 0.008^{Bc}	1483.73 ± 26.27^{Aab}
	7.5	5.18 ± 0.008^{Ca}	1.73 ± 0.008^{Ac}	1483.73 ± 26.27^{Aab}
	10	5.18 ± 0.008^{Ca}	1.53 ± 0.008^{Bd}	1483.65 ± 26.27^{Aab}
10.	1	4.77 ± 0.010^{Da}	2.00 ± 0.012^{Aa}	1256.08 ± 27.91^{Ba}
	2	4.76 ± 0.010^{Da}	1.99 ± 0.012^{Aa}	1234.18 ± 27.91^{Ba}
	3	4.77 ± 0.010^{Da}	1.97 ± 0.012^{Aab}	1148.06 ± 27.91^{Ba}
	4	4.76 ± 0.010^{Da}	1.94 ± 0.012^{Abc}	1201.72 ± 27.91^{BCa}
	5	4.88 ± 0.010^{Da}	1.91 ± 0.012^{Ac}	1223.38 ± 27.91^{Ba}
	7.5	4.89 ± 0.010^{Da}	1.83 ± 0.012^{Ad}	1234.18 ± 27.91^{Ba}
	10	4.90 ± 0.010^{Da}	1.76 ± 0.012^{Ae}	1234.41 ± 27.91^{Ca}
24.	1	4.24 ± 0.006^{Ea}	2.00 ± 0.006^{Aa}	1245.21 ± 27.85^{Ba}
	2	4.25 ± 0.006^{Ea}	1.98 ± 0.006^{Aa}	1212.67 ± 27.85^{Bab}
	3	4.27 ± 0.006^{Ea}	1.97 ± 0.006^{Aa}	1127.26 ± 27.85^{Bb}
	4	4.27 ± 0.006^{Ea}	1.92 ± 0.006^{Abc}	1116.47 ± 27.85^{Cb}
	5	4.30 ± 0.006^{Ea}	1.96 ± 0.006^{Aab}	1212.43 ± 27.85^{Bab}
	7.5	4.31 ± 0.006^{Ea}	1.89 ± 0.006^{Ac}	1278.06 ± 27.85^{Ba}
	10	4.32 ± 0.006^{Ea}	1.82 ± 0.006^{Ad}	1289.17 ± 27.85^{BCa}

*Capital letters represent the variation between different times in the proportion of each carbon source, while lowercase letters represent the variation between different carbon source proportions at each time.

CONCLUSION

In the food industry, antibacterial proteins known as enterocins, produced by enterococci, are gaining significant attention as alternatives to chemical food additives, aligning with consumer health concerns and demands. In this context, optimizing fermentation conditions is crucial for enhancing the cost-effective production of these bacteriocins in greater quantities. This study employed the one-factor-at-a-time (OFAT) method to investigate various commercial media, initial pH values, incubation temperatures, and carbon sources to determine the optimal conditions for producing mundticin-KS by

Enterococcus mundtii YB6.30. The highest yield of mundticin-KS was achieved by incubating the culture at 30°C for 6 to 8 hours in MRS broth supplemented with 1% (w/v) sucrose and an initial pH of 6.2. Future research should explore additional nutritional components or food-grade agricultural byproducts to further enhance cost-effective bacteriocin production by *E. mundtii* YB6.30.

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CONFLICT OF INTEREST
The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS
Selma Küçükçiftçi: investigation, data curation, writing – original draft; Burak Geniş: data curation, writing – review & editing; Yasin Tuncer: methodology, conceptualization, investigation, resources, supervision, writing – review & editing. All authors read and approved the final manuscript.

REFERENCES
Abbasiliasi, S., Tan, J.S., İbrahim, T.A.T., Ramanan, R.N., Vakhshiteh, F., Mustafa, S., Ling, T.C., Rahim, R.A., Ariff, A.B. (2012). Isolation of *Pediococcus acidilactici* Kp10 with ability to secrete bacteriocin-like inhibitory substance from milk products for applications in food industry. *BMC Microbiology,* 12: 260. https://doi.org/10.1186/1471-2180-12-260

Ahmad, V., Khan, M.S., Jamal, Q.M.S., Alzohairy, M.A., Karaawi, M.A., Siddiqui, S.D. (2017). Antimicrobial potential of bacteriocins: in therapy, agriculture and food preservation. *International Journal of Antimicrobial Agents,* 49(1): 1–11. https://doi.org/10.1016/j.ijantimicag.2016.08.016

Akpınar Kankaya, D., Tuncer, Y. (2020). Antibiotic resistance in vancomycin-resistant lactic acid bacteria (VRLAB) isolated from foods of animal origin. *Journal of Food Processing and Preservation*, e14468. https://doi.org/10.1111/jfpp.14468

Almeida-Santos, A.C., Novais, C., Peixe, L., Freitas, A.R. (2021). *Enterococcus* spp. as a producer and target of bacteriocins: a double-edged sword in the antimicrobial resistance crisis context. *Antibiotics*, 10: 1215. https://doi.org/10.3390/antibiotics10101215

Altınkaynak, T., Tuncer, Y. (2020). Fermente sucuktan izole edilen antilisterial *Enterococcus mundtii* YB6.30 tarafından üretilen bakteriyosinin karakterizasyonu. *Gıda/The Journal of Food*, 45(5): 963-976. https://doi.org/10.15237/gida.GD20081

Álvarez-Cisneros, Y.M., Ponce-Alquicira, E. (2019). Antibiotic resistance in lactic acid bacteria. In: *Antimicrobial Resistance - A Global Threat*. Kumar, Y. (ed), IntechOpen, http://dx.doi.org/10.5772/intechopen.80624.

Bayram, M., Yıldırım, Z. (2016). Beyaz peynirden bakteriyosin üreten bakterinin (*Enterococcus faecium*) izolasyonu ve bakteriyosinin karakterizayonu. *Gaziosmanpaşa Bilimsel Araştırma Dergisi*, 13: 103-115.

Bisht, V., Das, B., Navani, N.K. (2025). Bacteriocins sourced from traditional fermented foods for ensuring food safety: the microbial guards. *Journal of the Science of Food Agriculture*, https://doi.org/10.1002/jsfa.13783

Cladera-Olivera, F., Caron, G.R., Brandelli, A. (2004). Bacteriocin production by *Bacillus licheniformis* strain P40 in cheese whey using response surface methodology. *Biochemical Engineering Journal*, 21(1): 53-58. https://doi.org/10.1016/j.bej.2004.05.002

De Kwaadsteniet, M., Todorov, S.D., Knoetze, H., Dicks, L.M.T. (2005). Characterization of a 3944 Da bacteriocin, produced by *Enterococcus mundtii* ST15, with activity against Gram-positive and Gram-negative bacteria. *International Journal of Food Microbiology*, 105(3): 433-444. https://doi.org/10.1016/j.ijfoodmicro.2005.03.021

De Souza, E.L., de Oliveira, K.Á.R., de Oliveira, M.E.G. (2023). Influence of lactic acid bacteria metabolites on physical and chemical food properties. *Current Opinion in Food Science*, 49: 100981. https://doi.org/10.1016/j.cofs.2022.100981.

Feng, G., Guron, G.K.P., Churey, J.J., Worobo, R.W. (2009). Characterization of mundticin L, a class IIa anti-listeria bacteriocin from *Enterococcus mundtii* CUGF08. *Applied and Environmental Microbiology*, 75(17): 5708-5713. https://doi.org/10.1128/AEM.00752-09

Fguira, I.B., Fourati, Z., Kamoun, F., Tounsi, S., Jaoua, S. (2014). Isolation of the *Bacillus thuringiensis* plasmid carrying bacthuricin F4 coding genes and evidence of its conjugative transfer. *The Journal of Infection in Developing Countries*, 8(6): 727-732. https://doi.org/10.3855/jidc.3552

Franz, C.M.A.P., Van Belkum, M.J., Holzapfel, W.H., Abriouel, H., Gálvez, A. (2007). Diversity of enterococcal bacteriocins and their grouping in a new classification cheme. *FEMS Microbiology Reviews*, 31(3): 293-310. https://doi.org/10.1111/j.1574-6976.2007.00064.x

Garneau, S., Martin, N.I., Vederas, J.C. (2002). Two-peptide bacteriocins produced by lactic acid bacteria. *Biochimie*, 84(5-6): 577-592. https://doi.org/10.1016/s0300-9084(02)01414-1

Garsa, A.K., Kumariya, R., Sood, S.K., Kumar, A., Kapila, S. (2014). Bacteriocin production and different strategies for their recovery and purification. *Probiotics and Antimicrobial Proteins*, 6(1): 47-58. https://doi.org/10.1007/s12602-013-9153-z

Gök Charyyev, M., Özden Tuncer, B., Akpınar Kankaya, D., Tuncer, Y. (2019). Bacteriocinogenic properties and safety evaluation of *Enterococcus faecium* YT52 isolated from Boza, a traditional cereal based fermented beverage. *Journal of Consumer Protection and Food Safety*, 14(1): 41-53. https://doi.org/10.1007/s00003-019-01213-9

Graham, K., Stack, H., Rea, R. (2020). Safety, beneficial and technological properties of enterococci for se in functional food applications–a review. *Critical Reviews in Food Science and Nutrition*, 10: 1-26. https://doi.org/10.1007/s00003-019-01213-9

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

Jawan, R., Abbasiliasi, S., Tan, J.S., Mustafa, S., Halim, M., Ari, A.B. (2020). Influence of culture conditions and medium compositions on the production of bacteriocin-like inhibitory substances by *Lactococcus lactis* Gh1. *Microorganisms*, 8(10): 1454. https://doi.org/10.3390/microorganisms8101454

Karnwal, A., Malik, T. (2024) Exploring the untapped potential of naturally occurring antimicrobial compounds: novel advancements in food preservation for enhanced safety and sustainability. *Frontiers in Sustainable Food Systems*, 8: 1307210. https://doi.org/10.3389/fsufs.2024.1307210

Kasimin, M.E., Shamsuddin, S., Molujin, A.M., Sabullah, M.K., Gansau, J.A., Jawan, R. (2022). Enterocin: promising biopreservative produced by *Enterococcus* sp. *Microorganisms*, 10: 684. https://doi.org/10.3390/microorganisms10040684

Kawamoto, S., Shima, J., Sato, R., Eguchi, T., Ohmomo, S., Shibato, J., Horikoshi, N., Takeshita, K., Sameshima, T. (2002). Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. *Applied and Environmental Microbiology*, 68(8): 3830-3840. https://doi.org/10.1128/AEM.68.8.3830-3840.2002

Kuhan Sreedharan, D., Abbasiliasi, S., Mohamed, M.S., Ng, Z.J., Ariff, A.B., Lee, C.K., Tan, J.S. (2021). Fermentation strategies for improving the production of bacteriocin-like inhibitory substances by *Lactobacillus brevis* C23 with ntrient supplementation, pH, and temperature variations. *Journal of Food Processing and Preservation*, 45(11): 5914. https://doi.org/10.1111/jfpp.15914

Laranjo, M., Potes, M.E., Elias, M. (2019). Role of starter cultures on the safety of

fermented meat products. *Frontiers in Microbiology*, 10: 1–11. https://doi.org/10.3389/fmicb.2019.00853

Malheiro, P.S., Sant'Anna, V., Todorov, S.D., Franco, B.D.G.M. (2015). Optimization of growth and bacteriocin production by *Lactobacillus sakei* subsp. *sakei* 2a. *Brazilian Journal of Microbiology*, 46(1): 825-834. http://dx.doi.org/10.1590/S1517-838246320140279

Martínez-Bueno, M., Galvez, A.,Valdivia, E., Maqueda, M. (1990). A transferable plasmid associated with AS-48 production in *Enterococcus faecalis*. *Journal of Bacteriology*, 177(5): 2817-2818. https://doi.org/10.1128/jb.172.5.2817-2818.1990

Mendeş, M. (2019). İstatistiksel yöntemler ve deneme planlanması. Kriter Yayınevi, 636s., İstanbul.

Mohd Rasid, N.H., Abdul Halid, N., Song, A. A.-L., Sabri, S., Saari, N., Hasan, H. (2023). Effects of individual and combined fermentation factors on antimicrobial activity of nisin by *Lactococcus lactis* ATCC 11454. *Molecular Biotechnology*, 65: 861–870. https://doi.org/10.1007/s12033-022-00584-z

Miljkovic, M., Lozo, J., Mirkovic, N., O'Connor, P.M., Malesevic, M., Jovcic, B., Cotter, P.D., Kojic, M. (2018). Functional characterization of the lactolisterin BU gene cluster of *Lactococcus lactis* subsp. *lactis* BGBU1-4. *Frontiers in Microbiology*, 9: 2774. https://doi.org/10.3389/fmicb.2018.02774

Oliveira, F.S., da Silva Rodrigues, R., Cavicchioli, V.Q., de Carvalho, A.F., Nero, L.A. (2024). Influence of different culture media on the antimicrobial activity of *Pediococcus pentosaceus* ST65ACC against *Listeria monocytogenes*. *Brazilian Journal of Microbiology*, 55: 2539–2545. https://doi.org/10.1007/s42770-024-01391-1

Oliveira, M., Ferreira, V., Magalhães, R., Teixeira, P. (2018). Biocontrol strategies for mediterranean-style fermented sausages. *Food Research International*, 103: 438-449. https://doi.org/10.1016/j.foodres.2017.10.048

Öztürk, H., Geniş, B., Özden Tuncer, B., Tuncer, Y. (2023). Bacteriocin production and technological properties of *Enterococcus mundtii* and *Enterococcus faecium* strains isolated from sheep and goat colostrum. *Veterinary Research Communications*, 47: 1321–1345. https://doi.org/10.1007/s11259-023-10080-7

Parlindungan, E., Dekiwadia, C., Fones, O.A.H. (2021). Factors that influence growth and bacteriocin production in *Lactiplantibacillus plantarum* B21. *Process Biochemistry*, 107: 18–26. https://doi.org/10.1016/j.procbio.2021.05.009

Pattnaik, P., Grover, S., Batish, V.K. (2005). Effect of environmental factors on production of ichenin, a chromosomally encoded bacteriocin-like compound produced by *Bacillus licheniformis* 26L-10/3RA. *Microbiological Research*, 160(2): 213-218. https://doi.org/10.1016/j.micres.2005.01.006

Ren, B., Wu, W., Soladoye, O.P., Bak, K.H., Fu, Y., Zhang, Y. (2021). Application of biopreservatives in meat preservation: a review. *International Journal of Food Scince and Technology,* 56: 6124-6141. https://doi.org/10.1111/ijfs.15307

Reuben, R.C., Torres, C. (2024). Bacteriocins: potentials and prospects in health and agrifood systems. *Archives of Microbiology*, 206: 233. https://doi.org/10.1007/s00203-024-03948-y

Sabia, C., Manicardi, G., Messi, P., de Niederhäusern, S., Bondi, M. (2002). Enterocin 416K1, an antilisterial bacteriocin produced by *Enterococcus casseliflavus* IM 416K1 isolated from Italian sausages. *International Journal of Food Microbiology*, 75(1-1): 163-170. https://doi.org/10.1016/S0168-1605(01)00741-3

Sakoui, S., Derdak, R., Pop, O.L., Vodnar, D.C., Jouga, F., Teleky, B.-E., Addoum, B., Simon, E., Suharoschi, R., Soukri, A., El Khalfi, B. (2024). Exploring technological, safety and probiotic properties of *Enterococcus* strains: impact on rheological parameters in fermented milk. *Foods*, 13: 586. https://doi.org/10.3390/foods13040586

Saraiva, M.A.F., Birri, D.J., Brede, D.A., Baracat-Pereira, M.C., de Queiroz, V.M., Nes, I.F., de

Moraes, A.C. (2020). Nisin Z production by wild strains of *Lactococcus lactis* isolated from Brazilian (Italian Type) fermented sausage. *International Journal of Microbiology*, ID9309628. https://doi.org/10.1155/2020/9309628

Schelegueda, L.I., Vallejo, M., Gliemmo, M.F., Marguet, E.R., Campos, C.A. (2015). Synergistic antimicrobial action and potential application for fish preservation of bacteriocin produced by *Enterococcus mundtii* isolated from *Odontesthes platensis*. *LWT-Food Science and Technology*, 64(2): 794-801. https://doi.org/10.1016/j.lwt.2015.06.017

Sidek, N.L.M., Halim, M., Tan, J.S., Abbasiliasi, S., Mustafa, S., Ariff, A.B. (2018). Stability of bacteriocin-like inhibitory substance (BLIS) produced by *Pediococcus acidilactici* kp10 at different extreme conditions. *BioMed Research International*, 27: 5973484 https://doi.org/10.1155/2018/5973484

Tareq, I., Luti, K.J.K. (2022). Anapplication of bacteriocin-producing vaginal *Lactobacillus crispatus* IS30 in a gel formula against some vaginal pathogens. *Iraqi Journal of Science*, 63(2): 491-507. https://doi.org/10.24996/ijs.2022.63.2.7

Todorov, S.D., Dicks, L.M.T. (2009). Effect of modified MRS medium on production and purification of antimicrobial peptide ST4SA produced by *Enterococcus mundtii*. *Anaerobe*, 15(3): 65-73. https://doi.org/10.1016/j.anaerobe.2008.11.002

Todorov, S.D., Oliveira, R.P.S., Vaz-Velho, M. (2012). Media optimization of bacteriocin ST22Ch production by *Lactobacillus sakei* ST22Ch isolated from Salpicão, a traditional meat-product from Portugal. *Chemical Engineering Transactions*, 27: 283–288. https://doi.org/10.3303/CET1227048

Turgis, M., Vu, K.D., Millette, M., Dupont, C., Lacroix, M. (2016). Influence of environmental factors on bacteriocin production by human isolates of *Lactococcus lactis* MM19 and *Pediococcus acidilactici* MM33. *Probiotics and Antimicrobial Proteins*, 8(1): 53–59. https://doi.org/10.1007/s12602-015-9204-8

Valledor, S.J.D., Dioso, C.M., Bucheli, J.E.V., Park, Y.J., Suh, D.H., Jung, E.S., Kim, B., Holzapfel, W.H., Todorov, S.D. (2022). Characterization and safety evaluation of two beneficial, enterocin-producing *Enterococcus faecium* strains isolated from Kimchi, a Korean fermented cabbage. *Food Microbiology*, 102: 103886. https://doi.org/10.1016/j.fm.2021.103886

Yang, E., Fan, L., Yan, J., Jiang, Y., Doucette, C., Fillmore, S., Walker, B. (2018). Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria. *AMB Express*, 8: 10. https://doi.org/10.1186/s13568-018-0536-0

Zendo, T., Eungruttanagorn, N., Fujioka, S., Tashiro, Y., Nomura, K., Sera, Y., Kobayashi, G., Nakayama, J., Ishizaki, A., Sonomoto, K. (2005). Identification and production of a bacteriocin from *Enterococcus mundtii* QU2 isolated from soybean. *Journal of Applied Microbiology*, 99(5): 1181-1190. https://doi.org/10.1111/j.1365-2672.2005.02704.x