

**MICROBIAL CHARACTERIZATION OF VAN HERBY CHEESE USING  
MALDI-TOF MS AND EVALUATION OF STARTER CULTURE POTENTIAL  
OF SELECTED LACTIC ACID BACTERIA**

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**ABSTRACT**

This study examined the microbial and biochemical properties of Van Herby Cheeses, produced using traditional dry salting and brining methods. Biochemical analyses showed that dry salted cheeses contained 54.41±4.80% dry matter, 0.74±0.09 water activity, pH 5.11±0.76, 30.4±5.32% fat, and 5.81±1.20% salt, while brined cheeses had 62.60±3.20%, 0.81±0.03, 4.68±0.57, 29.0±8.22%, and 7.58±0.98%, respectively. Microbial identification using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) revealed a predominance of *Enterococcus*, *Lactobacillus*, *Leuconostoc*, and *Bacillus* species, as well as yeasts such as *Debaryomyces hansenii* and *Candida zeylanoides*. A total of 17 LAB isolates were assessed for acidification, proteolytic activity, diacetyl production, antibacterial effects, and antibiotic resistance. Among these, *E. faecium* S4<sub>31</sub> and *L. brevis* B1<sub>22</sub> exhibited strong acidifying capacity and notable antimicrobial properties, highlighting their promising potential as functional starter cultures for traditional cheese production.

**Keywords:** Van Herby Cheese, MALDI-TOF MS, microbiota, lactic acid bacteria, starter culture

**VAN OTLU PEYNİRİNİN MALDI-TOF MS YÖNTEMİ İLE MİKROBİYAL  
KARAKTERİZASYONU VE SEÇİLİ LAKTİK ASİT BAKTERİLERİNİN STARTER  
KÜLTÜR POTANSİYELİNİN DEĞERLENDİRİLMESİ**

**ÖZ**

Bu çalışmada, geleneksel yöntemle basma ve salamura olarak üretilen Van Otlı Peynirlerinin mikrobiyal ve biyokimyasal özelliklerinin tespit edilmesi ve izole edilen bazı laktik asit bakterilerinin

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(LAB) starter kültür olma potansiyelleri değerlendirilmiştir. Çalışmada basma ve salamura peynirlerin kuru madde, su aktivitesi, pH, yağ ve tuz içerikleri sırasıyla  $54.41 \pm 4.80$ -% $62.60 \pm 3.20$ ,  $0.74 \pm 0.09$ -% $0.81 \pm 0.03$ ,  $5.11 \pm 0.76$ -% $4.68 \pm 0.57$ ,  $30.4 \pm 5.32$ -% $29.0 \pm 8.22$  ve  $5.81 \pm 1.20$ -% $7.58 \pm 0.98$  olarak tespit edilmiştir. İzole edilen mikroorganizmalar MALDI-TOF MS yöntemi ile tür düzeyinde tanımlanmıştır. Örneklerde ağırlıklı olarak *Enterococcus*, *Lactobacillus*, *Leuconostoc* ve *Bacillus* cinslerine ait bakteri türleri belirlenmiş ve *Debaryomyces hansenii* ve *Candida zeylanoides* gibi maya türlerine de rastlanmıştır. Seçilen 17 LAB suşunun asidifikasyon yetenekleri, proteinaz ve diasetil üretim kapasiteleri ile antibakteriyel aktiviteleri ve antibiyotik duyarlılıkları değerlendirilmiştir. Özellikle *E. faecium* S4<sub>31</sub> ve *L. brevis* B1<sub>22</sub> suşları yüksek asidifikasyon ve antimikrobiyal aktiviteleriyle öne çıkmıştır. Çalışma sonuçları, Van Otlı Peynirinin çeşitlilik arz eden mikrobiyotasını ortaya koymakta ve izole edilen suşların starter kültür olarak kullanılabilceğini göstermektedir.

**Anahtar kelimeler:** Van Otlı Peynir, MALDI-TOF MS, mikrobiyota, laktik asit bakterileri, starter kültür

### INTRODUCTION

Cheese is a rich source of nutrients, with its high protein content (3–40%), as well as calcium, phosphorus, magnesium, zinc and vitamins A, B2, B6, B12. It is the dairy product with the most varieties (Alehosseini et al., 2025). Most cheeses are still produced according to local traditions and needs, and each has its unique flavour, aroma and texture (Kamber, 2007). These traditionally produced products are essential not only in social and cultural contexts but also in terms of economic impact, as they are considered value-added products (Serrano et al., 2024). Traditional dairy products in the world are not only a part of cultural heritage but also valuable in biotechnological and nutritional terms thanks to the environmental conditions specific to the region where they are produced, milk resources, local starter cultures, traditional production techniques and rich microbial diversity (Azzouz et al., 2024; Serrano et al., 2024). The cheese's microbial structure directly affects various cheese features, such as its aroma, texture, ripening profile, and safety (Coelho et al., 2022). In our country, with the 'Geographical Indication' granted by the Turkish Patent Office (TPO), traditional products produced with raw materials and production methods specific to a particular geographical region and thus have gained fame are protected (TPO, 2025). Van Herby Cheese is one of the seven regional products with a geographical indication patent belonging to Van province (Kırmacı and Kivik, 2024). Van Herby Cheese, which is produced in the Eastern Anatolia Region, especially in cities such as Diyarbakır, Muş, and Bitlis, is a cheese variety that

attracts attention with its natural aromatic herbs, raw sheep milk-based production, and traditional production method (Coşkun, 2024; Gülmez et al., 2024; Kırmacı and Kivik, 2024). However, sheep milk cannot be used alone in some productions; it can be used in a mixture with goat or cow milk. Herbs frequently used in the production of herb cheese belong to the *Liliaceae* (*Allium* sp.), *Lamiaceae* (*Thymus migricus*) and *Apiaceae* (*Ferula* sp., *Anthriscus nemorosa*) families. Herbs give the cheese its characteristic appearance and aroma, provide antimicrobial properties and shape the microbial profile (Çelik et al., 2008; Ocak et al., 2015a).

The production process of Van Herby Cheese begins with the straining of the milk and fermentation at a temperature similar to that of milking. After coagulation, the cheese is cut into small pieces, and herbs, especially wild garlic species, are added to the resulting pieces. Herbs are harvested in the spring season and are generally added to the cheese in two ways. Herbs are used fresh after being washed and sliced, or the herbs washed after harvest are placed in a brine containing 16% salt and can be added to the cheese after waiting for approximately 20 days (Çelik et al., 2007; Coşkun, 2024).

In recent years, studies on the microbiota of traditional cheeses produced in different geographical regions worldwide have increased. Portuguese Pico Cheese (Domingos-Lopes et al., 2017), Greek Graviera Cheese (Vandera et al., 2019), Iranian local cheeses (Barzegar et al., 2021), and Kazakh cheeses (Li et al., 2020) are some of the studies in this context. Microbial diversity

studies conducted on Turkish White and Herbal Cheeses have been addressed in various studies in the literature (İşleyici and Akyüz, 2009; Tunçtürk et al., 2014; Ocak et al., 2015a; Köse et al., 2021; Ekici et al., 2019; Coşkun, 2024; Oğuz and Andıç, 2024).

Accurate, rapid, and species-level identification of the microbiota in such traditional products is of great importance for product quality standardization, geographical indication studies, and the development of functional cultures. Among the new generation diagnostic technologies used for this purpose, the MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry) method stands out with its advantages, such as obtaining fast results, supporting culture-based analyses, and providing a high accuracy rate (Karasu Yalçın et al., 2021; Encu et al., 2022; Söylemez Milli et al., 2025).

This study examined 10 Van Herby Cheese samples obtained from Van province; biochemical and microbiological analyses of the samples and the identification of culturable microorganisms with the MALDI-TOF MS system were provided. In addition, the acid production capacity, proteinase activity, diacetyl production, antibacterial effect, and antibiotic susceptibility of the selected LAB strains were evaluated, and their potential starter culture properties were analyzed. Although there are microbiological studies on Van Herby Cheese are available in the literature, the first application of the MALDI-TOF MS method presents a unique approach to analyzing traditional products using modern techniques.

## MATERIALS AND METHODS

### Materials

#### *Herby cheese samples*

Ten herby cheeses, which were stated to be produced according to traditional methods and completed their ripening processes, were obtained from Van Cheese Bazaar, including dry salted herby cheese (n=5) and brined herby cheese (n=5). The dry salted cheese samples were coded with the letter B (B1, B2, B3, B4, B5), and

the brined cheese samples were coded with the letter S (S1, S2, S3, S4, S5). The samples were delivered to the laboratory in sterile containers in the cold chain. The cheeses were stored at +4 °C during the analyses. It is known that starter culture or pasteurization applications are not used in cheese samples produced with traditional methods. However, in these cheeses, where sheep, cow, and goat milk or their mixtures are used, fermentation is carried out with microbial rennet enzyme at around 32 °C after the raw milk is filtered. After this process, the curd is cut, some whey is removed, and local herbs are added to approximately 2% of the milk used. Dry salted cheeses are preserved by covering them with salt; brined cheeses are preserved in 14% (w/v) salt water (Ocak et al., 2015b; Coşkun, 2024).

#### *Bacteria*

Antimicrobial activities of strains isolated from cheeses were determined with microorganisms in the Culture Collection of Food Microbiology Laboratory, Department of Food Engineering, Bolu Abant İzzet Baysal University. In the study, *Escherichia coli* (*E. coli*) O157:H7 KUEN 1461 (Ankara University Food Engineering Culture Collection), *Bacillus cereus* (*B. cereus*) RSKK709, *Listeria monocytogenes* (*L. monocytogenes*) ATCC 7644, *Staphylococcus aureus* (*S. aureus*) ATCC 6538, and *Salmonella* Typhimurium (*S. Typhimurium*) ATCC 13076 were used as indicator microorganisms.

### Methods

#### *Determination of biochemical properties of herby cheeses*

In order to determine some biochemical properties of herby cheese samples, dry matter, salt, fat, pH, and water activity (aw) values were examined. pH measurements were determined electrometrically using a calibrated digital pH meter (Thermo Scientific™ Orion Star™ A211 Benchtop, USA). Salt content was measured according to the Mohr method (Nielsen, 2003; Köse et al., 2021); fat content (Gerber method) and dry matter amount were measured according to the method used by Case et al. (1985), and water activity (aw) was measured using a 25°C water activity meter (LabMaster, Novasina, Switzerland) (Nakagawa and Oyama, 2019). All analysis measurements were made in 2 replicates.

*Isolation of microorganisms from herby cheeses*

Cheese samples were brought to the laboratory under appropriate conditions, and 10 grams were taken under aseptic conditions and homogenized with 90 mL MRD (Maximum Recovery Diluent) for 2 minutes (AES Chemunex Smasher, BioMerieux, France). Homogenates were diluted to 10<sup>-6</sup> decimal dilution, and two parallel dilutions were cultured using the spread culture method. For total mesophilic aerobic bacteria (TMAB), incubation was performed on Plate Count Agar (PCA) at 32°C for 2-3 days, for coliform group bacteria on Violet Red Bile Agar (VRBA) at 37°C for 2 days, for yeast and mold on Yeast Extract Glucose Chloramphenicol (YGC) Agar at 28°C for 5-7 days, and lactic acid bacteria (LAB) on De Man, Rogosa and Sharpe (MRS) and M17 Agar at 37°C for 2-3 days (Halkman, 2019).

*Identification of isolates using MALDI-TOF MS BioTyper*

Herby cheese samples were cultured, and colonies developed in Petri dishes were examined after incubation. Morphologically different colonies were selected, and these microorganisms were identified using MALDI-TOF MS Autoflex Speed (Bruker Daltonics GmbH, Germany) BioTyper 3.4. Different colonies were analyzed using the extended direct transfer method specified by the manufacturer. The calibration of the method (MBT\_FC.par) was a thorough process, ensuring the highest level of accuracy, and was performed using BTS (Bacteria test standard, Bruker, Germany), containing eight different MS (mass spectrum) peaks in the m/z range of 2,000-20,000 Da.  $\alpha$ -CHCA (10 mg/mL) was used as the matrix, and the samples were transferred onto the MTP 384 Ground Steel Target with the matrix. MS signals for each sample were obtained in linear positive mode. Mass spectra were processed using BioTyper 3.4 software (Bruker Daltonics GmbH, Germany). The agreement between the spectra obtained from the isolates and the spectrum of the reference profile was expressed according to the scoring system of the BioTyper 3.4 software. The accuracy value of the score range was visualized as a colour scale (colours corresponding to the score value: 1.99;> green; >1.69 yellow; 0.0-1.69

red) (Söylemez Milli et al., 2025; Ertürkmen, 2025).

*Storage of strains*

After purity controls, the isolated strains were stored at -18°C in a medium containing 20% glycerol (Encu et al., 2022).

**Determination of some starter culture properties of strains**

*Percent acidity and acidification*

0.1 mL of the 18-hour active cultures of the isolates was inoculated into 10 mL of skim milk powder medium. Then, they were incubated at 37°C. At the 6th and 24th hours of incubation, 2 mL samples were taken from the inoculated medium and titrated with 0.1 N NaOH by adding 1-2 drops of phenolphthalein indicator. The lactic acid ratio was determined according to the amount of NaOH consumed in the titration (Kırmacı, 2010).

The pH value change over time determined the acidification level of the isolated strains. 0.1 mL of the active culture was inoculated into 10 mL of skim milk powder medium and incubated. At the 6th and 24th hours of incubation, 2 mL samples were taken, and their pHs were measured with a pH meter. pH changes are given as  $\Delta$ pH, and the evaluation of  $\Delta$ pH was made according to Ertürkmen and Öner (2015).

*Determination of proteinase activity*

The proteinase activity of the isolates was determined qualitatively. For this purpose, the medium was prepared by boiling 1% skim milk powder and 1.5% agarose in the microwave. Petri dishes were drawn so that 6 isolates were dropped into each dish. Then, 10  $\mu$ L of the active culture to be analyzed was dropped into the appropriate place on this medium and left for incubation at 37°C. At the 5th hour of incubation, the zone diameters formed in the Petri dishes were measured (Fox, 1989).

*Determination of antibacterial properties*

Antimicrobial activities of isolated microorganisms were determined by the spot cultivation method. First, approximately 1-2  $\mu$ L of

active cultures of the isolates to be tested were spot inoculated onto the MRS Agar surface and incubated at 37°C for 24 hours to ensure the isolates formed colonies. Then, 100 µL of pathogenic bacteria were mixed with 5 mL of Tryptic Soy Soft Agar (0.7% agar). Soft agar was poured onto the developed isolates to cover the Petri dish surface, and the Petri dishes were incubated at 37°C for 24 hours. At the end of incubation, the zone diameters formed around the colonies were measured in mm (Harris et al., 1989). Results with zone diameters greater than 2 mm were evaluated as positive (İşleröglü et al., 2008).

#### *Determination of diacetyl formation*

The diacetyl formation status of the isolates was determined qualitatively in a straightforward manner. For this purpose, 18–24-hour active cultures were inoculated into tubes containing 5 mL sterile skim milk powder medium. The tubes were incubated at 30°C for 24 hours. After incubation, 1 mL samples were taken from each tube, and 0.5 mL  $\alpha$ -naphthol (1% w/v) and KOH

(16% w/v) were added. Then, the solutions were incubated at 30°C for 1 minute, and the formation of red rings on the surface of the samples was evaluated as diacetyl production (Farahani et al., 2017).

#### *Determination of antibiotic susceptibility of strains*

The disk diffusion method determined the sensitivity of the isolated bacteria to antibiotics. For this purpose, the isolated microorganisms were spread on the MRS and M17 Agar surfaces with sterile swabs from 18–24-hour active cultures under aseptic conditions, covering the entire Petri surface. Then, antibiotic disks were placed in each Petri dish with 6 antibiotic disks (Liofilchem, Italy). The Petri dishes were left to incubate flat. The zones formed around the disks due to incubation were measured in mm (Charteris et al., 1998).

## RESULTS AND DISCUSSION

### Biochemical properties

Table 1 gives the dry matter, water activity ( $a_w$ ), pH, fat, and salt properties of the herby cheeses.

Table 1. Biochemical properties of Van Herby Cheeses

Sample	Dry Matter (%)	Water Activity ( $a_w$ )	pH	Fat (%)	Salt (%)
B1	54.7573	0.87	5.60	30.00	5.00
B2	53.3232	0.80	5.06	35.00	6.85
B3	55.6963	0.67	5.08	35.00	6.15
B4	47.4671	0.71	6.14	22.00	6.90
B5	60.8183	0.66	3.98	30.00	4.15
Average (B)	54.4124	0.74	5.17	30.40	5.81
S1	60.1725	0.82	4.73	20.00	7.50
S2	67.7925	0.78	5.53	35.00	9.00
S3	63.03	0.83	4.12	35.00	6.50
S4	59.76	0.78	4.86	35.00	8.00
S5	62.28	0.82	4.19	20.00	6.90
Average (S)	62.61	0.81	4.68	29.00	7.58

The dry matter percentage of the dry salted cheese samples was found as  $54.41 \pm 4.80$ ; water activity as  $0.74 \pm 0.09$ ; pH values as  $5.11 \pm 0.761$ ; fat percentage as  $30.4 \pm 5.32$  and salt percentage as  $5.81 \pm 1.20$ . The dry matter percentage of the Brined cheese samples was found as  $62.60 \pm 3.2$ ; water activity as  $0.81 \pm 0.03$ ; pH values as  $4.68 \pm 0.57$ ; fat percentage as  $29.0 \pm 8.22$  and salt percentage as  $7.58 \pm 0.98$ . Biochemical values

showed differences depending on the production type. Tunçtürk et al. (2014) evaluated the biochemical properties of Van Herby Cheese produced from cow, sheep, and goat milk and their mixtures. It was reported that there was no statistical difference in % dry matter, fat and salt values of cheeses produced from different milks at the end of 180 days of storage. Emirmustafaoğlu and Coşkun (2012) found the

dry matter content of herby cheese produced with goat milk as  $52.59 \pm 3.075\%$ , fat content as  $30.22 \pm 4.636\%$ , pH value as 4.68 and salt content as  $3.88 \pm 0.383$ . The amount of salt was determined to be less than the values we found in our study. However, other parameters are parallel to the results we found. The salt content ( $8.64 \pm 0.17\%$ ), pH ( $5.47 \pm 0.04$ ) and dry matter ( $60.37 \pm$

$0.39$ ) values found by Ekici et al. (2019) were also found to be similar to the results of our study.

**Microbiological properties**

Lactic acid bacteria (LAB), total mesophilic aerobic bacteria (TMAB), yeast mold and coliform bacteria were counted in herby cheeses. The results are given in Table 2.

Table 2. Microbial count results of Van Herby Cheeses

Sample	LAB (log CFU/g)	(log CFU/g)	Yeast-Mold (log CFU/g)	Coliform bacteria (log CFU/g)
S1	2.0	3.45	ND	ND
S2	4.68	4.63	3.41	ND
S3	2.47	ND	ND	ND
S4	6.91	6.66	ND	ND
S5	2.54	3.17	ND	ND
B1	6.6	4.8	ND	ND
B2	3.33	ND	ND	ND
B3	3.19	4.14	ND	ND
B4	6.17	6.57	ND	ND
B5	3.17	ND	6.83	ND

ND: Not detected/Found below detection limits

In S3, B3, and B5 samples, the TMAB was found to be below the detection limits. However, the yeast-mold count of the B5 sample was 6.83 log CFU/g, and that of the S2 sample was 3.41 log CFU/g. In other samples, the yeast-mold count was found below the detection limits. Coliform bacteria were not detected in any of the samples. The number of LAB in the dry salted cheese samples was found to be higher than the number of LAB in the brine-type samples. The high number of yeast mold in these samples may be due to the herbs used and the lack of hygienic conditions during production. Coşkun (2024) reported that some yeasts found in the brined herbs used in the production of herb cheese are tolerant to salt and sugar, can grow at low pH values, and species with low proteolytic activity can be used as a support starter. Coliform bacteria were not detected in any of the samples. Ocak et al. (2015b) also could not detect coliform group bacteria in Van Herby Cheese produced from pasteurized milk until the end of the ripening process. In Van Herby Cheese produced from raw milk, it was reported that the coliform bacteria count of the samples containing

$7.37 \pm 0.16$ ,  $6.96 \pm 0.17$  and  $6.92 \pm 0.11$  log CFU/g coliform bacteria at the beginning were below the detection limits at the end of storage (180 days). The number of LAB in the dry salted cheese samples was found to be higher than the number of LAB in the brined samples. The high salt content in the brined water in the brined cheeses may have penetrated the inner part of the cheeses and suppressed the development of microbiota. Since the salt was only coated on the outer part of the cheeses in the dry salted cheese samples, the microorganisms in the inner parts of the cheese samples may not have been affected by the high salt content. Ekici et al. (2019) reported the LAB count as  $3.70 \pm 0.17$  log CFU/g, coliform bacteria count as  $3.57 \pm 0.11$  log CFU/g, *Enterobacteriaceae* count as  $3.42 \pm 0.13$  log CFU/g, and *S. aureus* count as  $0.11 \pm 0.04$  log CFU/g in herby cheese samples (n = 100) obtained from Van province. The diversity and number of microorganisms contained in herby cheeses may vary depending on the production method of the samples, conditions in storage and sales places, microbial load and microbiota diversity of the milk used in the production of herby cheeses, antimicrobial

effects of different herbs added to herby cheese, and ripening process of the cheeses (Akkoç et al., 2018; Coşkun, 2024).

### Identification of isolates using MALDI-TOF MS BioTyper

The MALDI TOF-MS BioTyper 3.4 system identified different types of colonies in Petri dishes obtained from cultivations made from dry salted and brined-type cheeses. Accordingly, Table 3 gives the various genera and types of microorganisms isolated from the samples.

Table 3. MALDI-TOF MS results of microorganisms isolated from the cheeses

Samples	MALDI-TOF MS	Samples	MALDI-TOF MS
S1	<i>Enterococcus faecium</i> <i>Lactobacillus brevis</i>	B1	<i>Enterococcus faecium</i>
			<i>Lactobacillus brevis</i>
S2	<i>Bacillus mojavensis</i> <i>Debaryomyces hansenii</i> <i>Enterococcus durans</i> <i>Enterococcus faecium</i> <i>Kocuria varians</i> <i>Lactobacillus brevis</i>	B2	<i>Lactobacillus coryniformis</i>
			<i>Lactobacillus parabuchneri</i>
S3	<i>Enterococcus faecium</i> <i>Solibacillus silvestris</i>	B3	<i>Lactobacillus paracasei</i>
			<i>Pediococcus pentosaceus</i>
S4	<i>Enterococcus faecium</i>	B4	<i>Bacillus simplex</i>
			<i>Enterococcus faecalis</i>
S5	<i>Bacillus mojavensis</i> <i>Bacillus muralis</i> <i>Bacillus pumilus</i> <i>Bacillus subtilis</i> <i>Lactobacillus brevis</i>	B5	<i>Enterococcus faecium</i>
			<i>Lactobacillus curvatus</i>
			<i>Leuconostoc mesenteroides</i>
			<i>Paenibacillus amylolyticus</i>
			<i>Paenibacillus illinoisensis</i>
			<i>Bacillus clausii</i>
			<i>Bacillus pumilus</i>
			<i>Enterococcus faecium</i>
			<i>Paenibacillus amylolyticus</i>
			<i>Paenibacillus polymyxa</i>
			<i>Solibacillus silvestris</i>
			<i>Brachybacterium nesterenkovii</i>
			<i>Candida zeylanoides</i>
			<i>Debaryomyces hansenii</i>
			<i>Enterococcus durans</i>
			<i>Enterococcus italicus</i>
			<i>Lactobacillus paracasei</i>
			<i>Lactococcus lactis</i>
			<i>Leuconostoc mesenteroides</i>
			<i>Bacillus cereus</i>
			<i>Bacillus pumilus</i>
			<i>Enterococcus durans</i>
			<i>Enterococcus faecium</i>
			<i>Kocuria rhizophila</i>
			<i>Lactobacillus buchneri</i>
			<i>Paenibacillus amylolyticus</i>

As seen in Table 3, the microorganisms isolated from the samples are diverse. Figure 1 shows the

number of identified species in the calico and brined samples as a percentage.

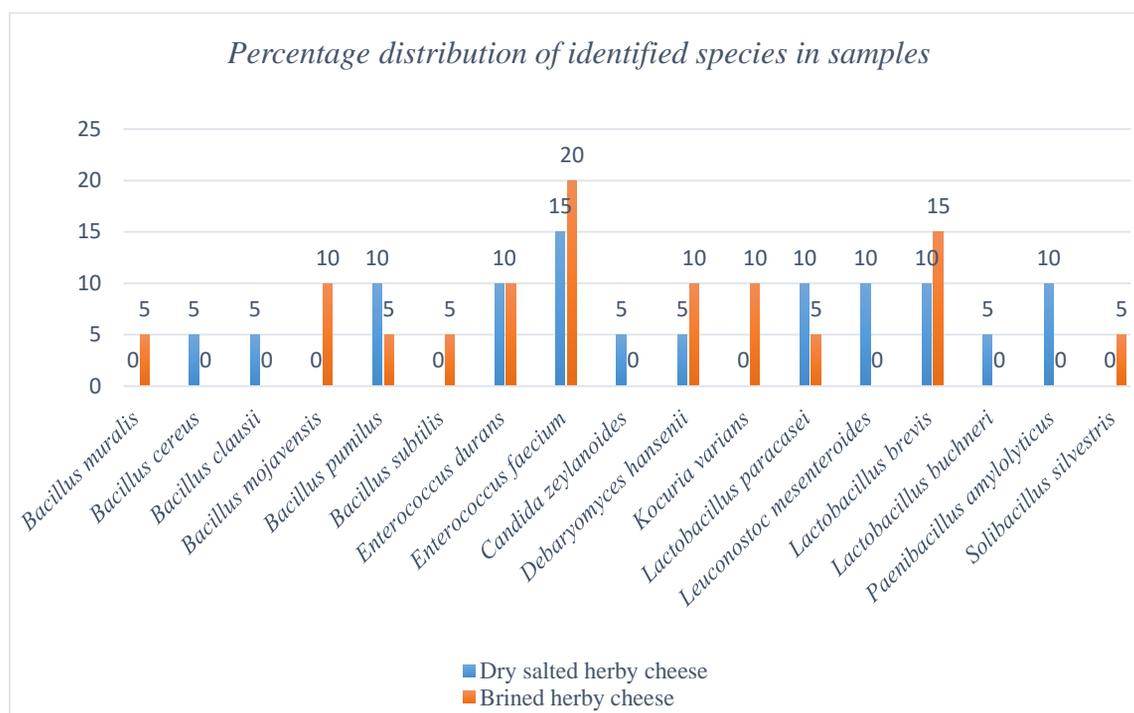


Figure 1. Percentage distribution of identified species in samples

The presence of LAB, especially *Enterococcus faecium* (*E. faecium*), was detected in the microbiota of the samples. However, it was observed that the *Bacillus* genus was dominant was the dominant member of the microbiota. When the results were examined, *Bacillus* spp., *Paenibacillus* spp., *Kocuria rhizophila* (*K. rhizophila*), *Brachybacterium nesterenkovi* (*B. nesterenkovi*), and LAB species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and yeast species *Candida zeylanoides* (*C. zeylanoides*), *Debaryomyces hansenii* (*D. hansenii*) were determined in the dry salted cheese samples. In the brined cheese samples, the presence of *Bacillus* spp., *Solibacillus* spp., *Enterococcus* spp., *Lactobacillus brevis* (*L. brevis*), *Kocuria varians* (*K. varians*) and yeast *D. hansenii* was detected. It was determined that LAB and *Bacillus* species were the dominant microbiota in the cheeses. *Bacillus* and *Paenibacillus* species can be easily isolated from plant roots, and it is known that *Paenibacillus* spp. has industrial importance (Gardener, 2004; Grady et al., 2016). Among the isolated *Bacillus* species, *B. clausii*, *B. subtilis* and *B. pumilus* stand out with their probiotic properties (Söylemez Milli et al., 2025). The habitats of *K. varians* and *K. rhizophila* are mammalian skin,

rhizosphere and soil (Al Bayatee and Alsammak, 2018). Therefore, these species are thought to originate from plant flora, animals or cheese producers. *B. nesterenkovi* was first isolated from dairy products and classified in 1992 (Gvozdyak et al., 1992). This species was not encountered in studies investigating the microbiota of Van Herby Cheese by species, culture-based or non-culture-based methods (Akyüz, 2009; Sudağidan et al., 2021; Rüstemoğlu et al., 2023). In this study, *B. nesterenkovi* was isolated from Van Herby Cheese for the first time and identified using the MALDI-TOF MS method. *D. hansenii* and *C. zeylanoides* may have survived throughout the shelf life of the herby cheese due to their tolerance to low temperatures and high salt concentrations (Mortensen et al., 2008). LAB isolated from cheeses may originate from natural sources such as raw milk, animal skin, cheese production equipment and environmental microbiota. Cultures used in traditional cheese production processes and cheese ripening play a role in forming flavour, taste, odour, aroma and texture. Lactic acid bacteria contribute to developing the sensory properties of cheese and preserving or even increasing its nutritional value (Coelho et al.,

2022). Dry salted cheeses were found to be richer in terms of LAB number and diversity than brined cheeses (Table 3, Figure 1). Cheese microbiota may vary depending on the cheese production period. Various factors, including pasteurization and milk cooling, curd cooking temperature, pH levels, salt content, cheese-making season and various other parameters, play a role. Cheese production season may also affect cheese microbiota (Azzouz et al., 2024). Sudağidan et al. (2021) determined the microbiota of Van Herby Cheese (n = 13) using 16S bacterial metagenomic analysis. It was found that *Lactobacillus*, *Lactococcus* and *Streptococcus* species were dominant in all cheeses. However, *Pediococcus*, *Vibrio*, *Enterococcus*, *Streptococcus*, and *Bacillus* species were also present in some cheeses. Similar to our study, *Enterococcus faecalis* (*E. faecalis*) was the dominant species determined at the species level in 2 cheeses. *Enterococcus* species constitute the secondary microbiota called non-starter lactic acid bacteria (NSLAB) in traditional cheeses and contribute to the aroma development during the ripening process of cheese (Aydin, 2017). In a study conducted on a different cheese type, Vandera et al. (2019) compared the microbiota of Graviera cheeses produced with commercial and traditional cultures. They found that *Enterococcus durans* (*E. durans*) and *E. faecium* species were the majority in traditionally produced cheeses, similar to our study. İşleyici and Akyüz (2009) isolated and identified the lactic acid bacteria found in Van herbed cheese. From the cheese samples, *Lactobacillus buchnerii* (*L. buchnerii*), *Lactobacillus brevis* (*L. brevis*), *Lactobacillus bulgaricus* (*L. bulgaricus*), *Lacticaseibacillus casei* (*Lcb. casei*), *Lactobacillus delbrueckii* (*L. delbrueckii*), *Limosilactobacillus fermentum* (*Lim. fermentum*), *Lactobacillus helveticus* (*L. helveticus*), *Lactobacillus lactis* (*L. lactis*), *Lactiplantibacillus plantarum* (*Lpb. plantarum*), *Lactobacillus pseudoplantarum* (*L. pseudoplantarum*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Lactobacillus tolerance* (*L. tolerance*), *Leuconostoc* spp., *Pediococcus* spp., *E. faecalis*, *E. faecium*, *L. lactis*, *Lactococcus diacetyllactis* (*L. diacetyllactis*) and *Lactococcus cremoris* (*L. cremoris*) species were isolated. The isolated bacteria are similar to those in our study. Rüstemoğlu et al. (2023) determined the microbiota of herby

cheeses from the Şırnak region using Next Generation Sequencing (NGS) technology. The study found that *Lactobacillaceae* and *Streptococcaceae* were the dominant genera in the microbiota of the herby cheese analyzed. At the species level, it was determined that the most dominant species in 16 herby cheese samples were *Companilactobacillus ginsenosidimutans* (*C. ginsenosidimutans*) and *L. delbrueckii*. *Enterococcaceae* was determined at high levels in 6 of the cheeses. In the study, *C. ginsenosidimutans*, *Weissella jogaejeotgali* (*W. jogaejeotgali*) and *Lactobacillus koreensis* (*L. koreensis*) species were identified and reported for the first time in a dairy product.

#### Determination of starter culture characteristics

The isolated strains were taken as stock, some lactic acid bacteria were selected according to their viability levels after storage, and the ability of these isolates to be starter cultures was examined. Bacteria belonging to the genera *Lactobacillus* (3 *L. brevis*, 2 *L. coryniformis*, 1 *L. parabuchneri* and 1 *L. buchneri*) and *Enterococcus* (5 *E. faecium*, 4 *E. durans*, and 1 *E. faecalis*) were selected from the cultures. The acid production capacities of these bacteria, antimicrobial activities against some pathogens, proteinase activities and diacetyl production abilities were determined. In addition, the resistance of these strains to various antibiotics was determined.

#### Percent acidity and acidification

The acid production of the isolates in terms of lactic acid in the skim milk powder medium and the medium's pH were measured and given in Table 4.

When the lactic acid production levels of the strains were examined, the strain that produced the most acid was *E. faecium* S4<sub>31</sub>, and the strain that produced the least acid was *L. buchneri* B5<sub>02</sub>. When the  $\Delta$ pH values of the strains at the end of 6 hours were examined, it was seen that *L. brevis* strains and *L. buchneri* B5<sub>02</sub> strain showed low acidification. Of the *L. brevis* strains, S2<sub>11</sub> showed high acidification at the end of 24 hours. According to the  $\Delta$ pH values of the strains at the end of 6 hours, only *E. faecium* S4<sub>31</sub> and *E. durans*

S2<sub>13</sub> showed high acidification, while the other strains showed moderate acidification. When the ΔpH<sub>24</sub> values of the strains were examined, only *L. brevis* B1<sub>22</sub>, *L. parabuchneri* B1<sub>39</sub>, and *L. buchneri* B5<sub>02</sub> had low acidification levels. When the lactic acid production levels of the strains are examined, it is seen that they vary between 0.072% (*L. buchneri* B5<sub>02</sub>) and 0.135% (*E. faecium* S4<sub>31</sub>). Lactic acid bacteria's most important feature and function is lactose fermentation in milk (Li et al., 2020). When the ΔpH values of the strains are examined, only *L. brevis* B1<sub>22</sub>, *L. parabuchneri* B1<sub>39</sub>, and *L. buchneri* B5<sub>02</sub> remained at low acidification

levels at the end of 24 hours, while all strains showed high acidification. Considering the ripening process of the cheese, the high acidification of the cultures in the first 24 hours was found to be positive in this respect. Bozoudi et al. (2015) stated that the *Enterococcus* strains they isolated from Feta cheese showed low acidification, unlike our study. *E. faecium* S4<sub>31</sub> and *E. durans* S2<sub>13</sub> showed high acidification after 6 hours in our study. Silva et al. (2023) similarly reported that most species (57%) of *Enterococcus* strains were able to reduce the pH of milk to ≤5 within 18 h of fermentation.

Table 4. Percent acidity and acidification values of isolates

Isolate name	Lactic acid production (%)	Acidification	
		ΔpH 6	ΔpH 24
<i>L. coryniformis</i> B1 <sub>19</sub>	0.117	1.37	2.14
<i>L. brevis</i> B1 <sub>22</sub>	0.126	0.89	0.96
<i>L. coryniformis</i> B1 <sub>23</sub>	0.117	1.22	2.0
<i>L. parabuchneri</i> B1 <sub>39</sub>	0.108	1.05	1.49
<i>E. faecalis</i> B2 <sub>02</sub>	0.144	1.31	1.99
<i>E. faecium</i> B2 <sub>04</sub>	0.081	1.33	2.36
<i>E. faecium</i> B3 <sub>07</sub>	0.126	1.25	2.36
<i>E. durans</i> B4 <sub>33</sub>	0.126	1.42	2.42
<i>E. durans</i> B5 <sub>01</sub>	0.126	1.45	2.4
<i>L. buchneri</i> B5 <sub>02</sub>	0.072	0.89	0.97
<i>L. brevis</i> S1 <sub>11</sub>	0.135	0.87	2.29
<i>E. faecium</i> S1 <sub>12</sub>	0.135	1.45	2.53
<i>L. brevis</i> S2 <sub>11</sub>	0.099	0.86	2.0
<i>L. brevis</i> S2 <sub>12</sub>	0.099	0.7	1.1
<i>E. durans</i> S2 <sub>13</sub>	0.153	1.54	2.59
<i>E. faecium</i> S3 <sub>02</sub>	0.117	1.09	2.03
<i>E. faecium</i> S4 <sub>31</sub>	0.135	1.57	2.66

### Antimicrobial activity

The antimicrobial activity of the isolates against pathogens found in the laboratory culture collection was tested, and the results obtained are given in Table 5.

Eight strains showed different levels of antimicrobial activity against the pathogens used in the study. Only *E. faecalis* B2<sub>02</sub> was effective against *Listeria monocytogenes*; only *L. brevis* S1<sub>11</sub> was effective against *S. aureus*, *E. faecium* S3<sub>02</sub> strain

showed antimicrobial activity against *B. cereus*, *E. coli* O157:H7 and *S. Typhimurium*. Primary metabolites, such as lactic acid, ethanol, carbon dioxide, as well as bacteriocin production, are the characteristics responsible for the antimicrobial activity of LAB species (Khushboo et al., 2023). Champiri et al. (2022) showed antimicrobial activity against *L. brevis*, *S. Typhimurium*, *S. aureus*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *E. faecalis* isolated from local cheeses. In our study, *L. brevis* S2<sub>11</sub> isolated showed antimicrobial activity against

*B. cereus* and *E. coli* O157:H7; *L. brevis* S1<sub>11</sub> showed antimicrobial activity against *S. aureus*, similar to the results of Champiri et al. (2022). Other *L. parabuchneri*, *E. faecalis*, *E. faecium*, and *E. durans* species we isolated also showed different levels of antimicrobial activity against the pathogens used in the study. Barzegar et al. (2021) determined the antimicrobial activity of *Lactobacillus* species isolated from Iranian cheese made from raw milk.

Isolated *L. brevis* B2 and B3 species showed antimicrobial activity against *E. coli*, *S. Typhimurium*, *P. aeruginosa*, and *S. aureus*. In another study, *Enterococcus* spp. strains isolated from Turkish and Iranian white cheeses showed antimicrobial effects against *E. coli*, *Salmonella* Enteritidis, and *S. Typhimurium* (Hajikhani et al., 2021).

Table 5. Inhibition diameter of isolates against pathogens (mm)

Isolate name	<i>L. monocytogenes</i>	<i>B. cereus</i>	<i>E. coli</i> O157:H7	<i>S.</i> Typhimurium	<i>S. aureus</i>
<i>L. coryniformis</i> B1 <sub>19</sub>	-	-	-	-	-
<i>L. brevis</i> B1 <sub>22</sub>	-	-	-	-	-
<i>L. coryniformis</i> B1 <sub>23</sub>	-	-	-	-	-
<i>L. parabuchneri</i> B1 <sub>39</sub>	-	-	-	16	-
<i>E. faecalis</i> B2 <sub>02</sub>	11	-	-	-	-
<i>E. faecium</i> B2 <sub>04</sub>	-	-	-	16	-
<i>E. faecium</i> B3 <sub>07</sub>	-	-	-	-	-
<i>E. durans</i> B4 <sub>33</sub>	-	-	-	-	-
<i>E. durans</i> B5 <sub>01</sub>	-	-	-	-	-
<i>L. buchneri</i> B5 <sub>02</sub>	-	-	-	-	-
<i>L. brevis</i> S1 <sub>11</sub>	-	-	-	-	11
<i>E. faecium</i> S1 <sub>12</sub>	-	-	-	18	-
<i>L. brevis</i> S2 <sub>11</sub>	-	16	13	-	-
<i>L. brevis</i> S2 <sub>12</sub>	-	-	-	-	-
<i>E. durans</i> S2 <sub>13</sub>	-	17	-	-	-
<i>E. faecium</i> S3 <sub>02</sub>	-	12	12	14	-
<i>E. faecium</i> S4 <sub>31</sub>	-	-	-	-	-

-: Inhibition zone could not be detected

### Proteinase activity

The proteinase activity of the isolates was determined by measuring the zone diameter formed in the medium prepared with 1% skimmed milk powder. The results obtained are given in Table 6.

As shown in Table 6, only 7 of the isolates exhibited proteinase activity. Proteolytic activity is effective in the degradation of protein in cheese and flavour formation. However, it indirectly contributes to bacterial growth and competitive advantage, as well as to probiotic effect and texture (Kieliszek et al., 2021). However, high levels of proteolysis cause a bitter taste in cheese. Proteolysis is affected by the concentrations of rennet and milk proteinases and production

conditions (Şimşek et al., 2016). Except for *Enterococcus* spp. and *L. brevis* S2<sub>11</sub> strains isolated in our study, it was found that other strains showed proteinase activity. Lim et al. (2019) qualitatively determined the ability of 17 LAB cultures isolated from Malaysian fermented foods to produce and secrete extracellular proteolytic enzymes using the skim milk agar hydrolysis test as in our study. At the end of the analysis, a clear hydrolysis zone was observed in skim milk agar. This result shows that the tested isolates can produce and secrete extracellular proteolytic enzymes for hydrolyzing whitish opaque casein molecules into colorless peptide fragments. Albayrak and Duran (2021) reported that *Enterococcus* strains isolated from white cheeses did not show proteolytic activity. In contrast, Mercha

et al. (2020) reported that *E. durans* strains isolated from camel milk had proteolytic activity, and proteolytic zone diameters ranged from  $15.33 \pm 0.28$  mm to  $20.50 \pm 0.50$  mm.

Table 6. Zone diameters of isolates (mm)

Isolate name	Zone diameter
<i>L. coryniformis</i> B1 <sub>19</sub>	10
<i>L. brevis</i> B1 <sub>22</sub>	10
<i>L. coryniformis</i> B1 <sub>23</sub>	8
<i>L. parabuchneri</i> B1 <sub>39</sub>	12
<i>E. faecalis</i> B2 <sub>02</sub>	-
<i>E. faecium</i> B2 <sub>04</sub>	-
<i>E. faecium</i> B3 <sub>07</sub>	-
<i>E. durans</i> B4 <sub>33</sub>	-
<i>E. durans</i> B5 <sub>01</sub>	-
<i>L. buchneri</i> B5 <sub>02</sub>	9
<i>L. brevis</i> S1 <sub>11</sub>	10
<i>E. faecium</i> S1 <sub>12</sub>	-
<i>L. brevis</i> S2 <sub>11</sub>	-
<i>L. brevis</i> S2 <sub>12</sub>	9
<i>E. durans</i> S2 <sub>13</sub>	-
<i>E. faecium</i> S3 <sub>02</sub>	-
<i>E. faecium</i> S4 <sub>31</sub>	-

-: No proteolytic zone

### Diacetyl formation

It was determined qualitatively whether the isolates produced diacetyl. Of the 17 isolated strains whose starter culture properties were examined, *L. coryniformis* B1<sub>19</sub> and *L. coryniformis* B1<sub>23</sub> produced good levels of diacetyl. *L. buchneri* B5<sub>02</sub>, *E. faecium* B3<sub>07</sub>, *L. brevis* B1<sub>22</sub>, *E. faecium* B2<sub>04</sub>, *L. brevis* S1<sub>11</sub>, and *L. brevis* S2<sub>11</sub> did not produce diacetyl. Other strains produced moderate levels of diacetyl. Diacetyl is a naturally occurring compound in cheeses responsible for forming a butter-like aroma. However, high levels of diacetyl production can lead to a bitter taste in cheese. In addition, the produced diacetyl can convert to acetone depending on the conditions during storage. Therefore, the storage period should be optimized, together with high-concentration diacetyl production at the end of fermentation (Kim et al., 2021). Nicosia et al. (2023) qualitatively determined the proteolytic activity and diacetyl production of 26 LAB strains isolated from Italian and Brazilian cheeses, whose potential for use in cheese production was

investigated, as in our study. In the study, it was reported that proteases hydrolyze caseins by forming peptides that are further broken down into amino acids, and the resulting amino acids are converted into volatile and non-volatile compounds that play an important role in the sensory properties of cheese through specific catabolic pathways. Of the isolates studied, *L. lactis*, *L. delbrueckii*, and *L. rhamnosus*, which were found to have high diacetyl production, high proteolytic activity, and suitable other starter culture characteristics, were selected as the most promising strains for use as adjunct cultures. Domingos-Lopes et al. (2017) stated that 87% of lactic acid bacteria isolated from Pico cheese produced diacetyl and that the ability to produce diacetyl from citrate depended on the species and strain. 60% of *Leuconostoc* spp., 33% of *Lactococcus* spp., 82% of *Lactobacillus* spp., and 92% of *Enterococcus* spp. produced diacetyl. Diacetyl production was observed in most *L. paracasei*, *Lpb. plantarum* and *E. faecalis* strains in the study.

### Antibiotic susceptibility of isolates

Table 7 presents the strains' susceptibilities to the antibiotics used in the study, along with the antibiotic action mechanisms.

The antibiotic resistance of starter cultures or probiotics does not pose a safety concern except for resistance phenotypes resulting from mutations or natural resistance mechanisms. Some probiotics with natural antibiotic resistance can be used to strengthen the intestinal microbiota after antibiotic treatment. However, transferring some resistance genes to pathogenic bacteria poses a serious safety problem (Gueimonde et al., 2013). In our study, the susceptibility of the isolates to 15 different antibiotics was examined (Table 7). All *Lactobacillus* and *Enterococcus* isolates were found to be resistant to ofloxacin, nalidixic acid, and gentamicin except *L. brevis* B1<sub>22</sub> and susceptible to chloramphenicol. In addition, all *Lactobacillus* isolates were found to be susceptible to ampicillin, erythromycin (except *L. coryniformis* B1<sub>19</sub>), and chloramphenicol and resistant to vancomycin. Encu et al. (2022) found that lactic acid bacteria isolated from yoghurt produced by traditional

methods were susceptible to ampicillin, erythromycin, chloramphenicol, clindamycin, rifampicin, cefotaxime, streptomycin, teicoplanin, tetracycline, and vancomycin; and resistant to gentamicin, nalidixic acid, ofloxacin, ciprofloxacin, and trimethoprim-

sulfamethoxazole. This result is similar to our findings. Domingos-Lopes et al. (2017) reported that 95% of the isolates were resistant to nalidixic acid; enterococci were resistant to chloramphenicol at a level of 2%.

Table 7. Antibiotic susceptibility status of isolates

Isolate name	Antibiotics by mechanism of effect														
	Inhibition of cell wall synthesis		Inhibition of DNA gyrase enzyme			RNA polymerase inhibition	Inhibition of cell wall synthesis		Inhibition of protein synthesis		Inhibition of ribosome 50S subunit		Inhibition of folate synthesis		Inhibition of protein synthesis
	CTX30	AMP10	CIP5	NA30	OFX5	RD5	VA5	TEC30	S300	CN10	E15	CD2	TM5	SXT25	C30
<i>L. coryniformis</i> B119	R	S	S	R	R	R	R	I	S	R	R	I	R	I	S
<i>L. brevis</i> B122	S	S	R	R	R	S	R	R	I	S	S	R	S	S	S
<i>L. coryniformis</i> B123	R	S	I	R	R	R	R	S	I	R	S	R	S	S	S
<i>L. parabuchneri</i> B139	S	S	R	R	R	S	R	R	I	R	S	S	S	R	S
<i>E. faecalis</i> B202	R	S	R	R	R	R	R	R	R	R	I	R	I	I	S
<i>E. faecium</i> B204	S	S	R	R	R	S	S	S	R	R	R	S	R	R	S
<i>E. faecium</i> B307	S	S	R	R	R	S	S	S	S	R	S	R	R	R	S
<i>E. durans</i> B433	S	S	S	R	R	S	S	S	I	R	S	S	R	R	S
<i>E. durans</i> B501	R	S	I	R	R	S	R	I	I	R	S	S	S	S	S
<i>L. buchneri</i> B502	R	S	R	R	R	S	R	R	S	R	S	S	R	R	S
<i>L. brevis</i> S111	S	S	R	R	R	S	R	R	I	R	S	R	S	R	S
<i>E. faecium</i> S112	R	R	I	R	R	R	S	S	S	R	R	R	R	R	S
<i>E. durans</i> S211	R	R	I	R	R	R	S	S	R	R	S	R	R	R	S
<i>L. brevis</i> S212	S	S	R	R	R	S	R	R	R	R	S	I	S	S	S
<i>E. durans</i> S213	S	R	R	R	R	S	S	S	R	R	R	S	R	R	S
<i>E. faecium</i> S302	S	R	I	R	R	S	S	S	S	R	R	R	R	R	S
<i>E. faecium</i> S431	S	R	I	R	R	S	S	S	R	R	R	S	R	R	S

S: Susceptible, I: Intermediately susceptible, R: Resistant (Ampicillin 10 µg, OFX5: Ofloxacin 5 µg, RD5: Rifampicin 5 µg, VA5: Vancomycin 5 µg, S300: Streptomycin 300 µg, NA30: Nalidixic acid 30 µg, TM5: Trimethoprim 5 µg, E 15: Erythromycin 5 µg, C30: Chloramphenicol 30 µg, TEC30: Teicoplanin 30 µg, CIP5: Ciprofloxacin 5 µg, SXT25: Trimethoprim sulfamethoxazole 25 µg, CD2: Clindamycin 2 µg, CTX30: Cefotaxime 30 µg, CN10: Gentamicin 10 µg)

The study results are similar to our findings. Domingos-Lopes et al. (2017) stated that vancomycin is a last-resort antibiotic for treating infections. Therefore, vancomycin-resistant enterococci are a source of concern regarding acquired antibiotic resistance. In our study, *E. durans* B501, one of the *Enterococcus* strains isolated, was found to be resistant to vancomycin (Table 7). Gad et al. (2014) found that *Lactobacillus* strains isolated from milk and pharmaceutical products

were resistant to vancomycin, similar to our study. Unlike our study, Nalepa and Markiewicz (2022) found that 12% of LAB isolated from cheeses produced from cow, sheep, and goat milk were resistant to tetracycline, erythromycin, and chloramphenicol. The study determined that only 5 of the 31 LAB isolates that were phenotypically sensitive to all antibiotics tested did not carry antibiotic resistance genes. It was emphasized that this result revealed that LAB found in local

cheeses had acquired antibiotic-resistance genes and posed a potential threat.

### CONCLUSION

This study is one of the first to characterize the microbial composition of Van Herby Cheese produced using traditional methods, with the aid of the modern MALDI-TOF MS method. The results provide important insights into the rich and unique microbiota of this cheese, and contributing to an understanding of the technological and bifunctional potential of microorganisms used in traditional dairy products. Acid production capacity and antimicrobial activities against some pathogenic microorganisms of the *Lactobacillus* and *Enterococcus* strains isolated within the scope of the study were evaluated.

In particular, *E. faecium* S4<sub>31</sub> and *L. brevis* B1<sub>22</sub> are considered candidates for starter cultures due to their strong acidification capacities and demonstrated antimicrobial effects. Special starter culture formulations, developed with combinations of these strains, can be integrated into traditional production processes, thereby shortening the fermentation period and increasing the product's shelf life. The antimicrobial properties of lactic acid bacteria play a crucial role in preventing microbial contamination in cheese. To further enhance the effectiveness of these strains against pathogenic bacteria, the use of additional natural antimicrobial compounds (e.g. nisin or other peptides) can be explored. This may be useful in improving the cheese's hygienic quality and providing a safer product in terms of health. However, it has been observed that some isolates exhibited a resistance profile to antibiotics. This situation necessitates a detailed evaluation of the safety of these microorganisms before they are used as starter cultures. In this context, the presence of antimicrobial resistance genes, particularly, should be carefully considered in both public health and probiotic applications.

The study's findings reveal that local microbial resources can be effectively utilized in controlled fermentation processes, thereby ensuring

standardization in traditional products. Future studies should focus on evaluating the technological performance of these strains under real-world production conditions, their impact on sensory quality, and their commercialization potential. In addition to protecting the microbial heritage of Van Herby Cheese, creating genetically and technologically defined local strain collections is strategically essential in sustainable production and supporting geographically indicated products.

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### DECLARATION OF INTEREST

The authors of this article have no conflict of interest with other individuals or institutions.

### AUTHOR CONTRIBUTIONS

All authors contributed to the analysis and writing of the article and read and approved the final version.

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