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Technological potential of native lactic acid bacteria isolated from Swiss-type artisanal cheese (Ancash, Peru) for their application in food

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Swiss-type artisanal cheese is highly appreciated sensorially, its flavor is often associated with the lactic acid bacteria involved in its production, which in many cases are indigenous. Three artisanal Swiss-type cheeses of greatest preference in the market of Huaraz (Ancash) were selected. The main LAB were isolated and identified and their safety properties and technological potential for their application in new cheeses were verified *in vitro* and evaluated by consumers. Eleven strains were confirmed as LAB by Gram-positive and catalase-negative biochemical tests; according to 16S rDNA, seven strains belonged to *Lacticaseibacillus paracasei* (KQ3, EQ1, CQ1, YQ1, LQ2, GQ2 and TQ1), three strains to *Lentilactobacillus parabuchneri* (BQ2, OQ2 and RQ3), and one to *Lactiplantibacillus* sp. (QQ3). In safety assays, LAB did not exhibit gelatinase or hemolytic activities. In addition, *L. paracasei* KQ3, GQ2 and *L. parabuchneri* BQ2 effectively inhibited pathogens such as *S. aureus*, *E. coli* and *L. monocytogenes*. Antibiotic susceptibility was variable among strains. *L. paracasei* CQ1, EQ1, KQ3, TQ1 and *Lactiplantibacillus* sp. QQ3 showed high milk acidification capacity (0.16-1.44%) and reduced pH from 6.6 to 3.5 after 72 h of incubation. *L. paracasei* CQ1, *Lactiplantibacillus* sp. QQ3 and *L. paracasei* KQ3 showed the highest casein degradation zones (20.8-11.5 mm). All strains showed lipolytic activity, with *Lactiplantibacillus* sp. QQ3, *L. paracasei* CQ1 and *L. parabuchneri* BQ2 standing out with halos of 30.8-36.3 mm. *Lactiplantibacillus* sp. QQ3 and *L. paracasei* TQ1 showed ability to produce diacetyl. The best strains were tested in cheese production where *L. paracasei* CQ1 showed the best sensory qualities. Finally, the native BAL strains showed a high potential for the production of natural, safe and sensorially acceptable dairy products.

KEYWORDS

protease, lipase, aromatic compounds, antibiotic resistance, molecular identification

1 Introduction

Lactic acid bacteria (LAB) involved in cheese making can determine its physicochemical and sensory qualities (Afshari et al., 2020). Based on their properties and their role in the fermentation process, LAB are classified into two groups: (i) starter strains, which accelerate fermentation due to their ability to produce high levels of lactic acid (De Melo Pereira et al., 2020; Zarzecka et al., 2020), and (ii) adjunct or non-starter lactic cultures, which are involved in improving the flavor and aroma during the cheese ripening process (Baptista et al., 2018; Levante et al., 2020a).

There are about 2,000 varieties of cheese in the world (Berno and Fusté-Forné, 2020). In addition, each cheese can contain up to 100,000 million bacteria per gram (Kilcawley, 2016). These microorganisms use the carbohydrates, proteins and lipids of the milk, producing various volatile compounds and contributing to the good taste of the final products (Kilcawley, 2016; Blaya et al., 2018). Among the diversity of cheese, the Swiss type is a hard or semi-hard cheese whose interior structure has holes called "eyes"(Hu, 2012; Fröhlich-Wyder et al., 2017) and includes some subtypes like Emmental, Comte, Maasdam, Beauford, Grevé, Gruyère français, Appenzeller, Tilsit, and others (Fröhlich-Wyder et al., 2013, 2017; Hayaloglu, 2016; Wolf et al., 2016). Although there is no international definition for the Swiss type cheese, it is identified by lactic fermentation in its first stage of elaboration, followed by propionic fermentation (Hu, 2012; Cheng, 2013). Its preparation includes the addition of *Streptococcus thermophilus* as a starter strain that reduces the pH and induces the growth of inoculated *Lactobacillus helveticus* (Baptista et al., 2018). The proteinases and peptidases produced by these lactobacilli play an important role in the breakdown of casein during cheese fermentation and ripening, contributing to the synergistic growth of the strains of *S. thermophilus* and *L. helveticus* (Hayaloglu, 2016) and allowing the development of the flavor (Cao et al., 2021). In addition, propionic fermentation is responsible for the holes inside the cheese through CO_2 production and its mild flavor (Hu, 2012; Fox and Guinee, 2013).

Taxonomic identification of the cheese microbiota generally uses physiological, phenotypic and biochemical methods, but species levels are sometimes not reached (Hassanzadazar et al., 2017; Sharma et al., 2020). Molecular tools, such as 16 s rDNA sequencing, are applied to taxonomically identify the cheese-associated bacterial species (Hejazi et al., 2019; Hajigholizadeh et al., 2020). The unknown diversity of LAB in artisanal cheese and other fermented foods is attractive to the food industry because native bacterial species could be used to improve the nutritional, technological, and safety characteristics of different foods, also increasing their shelf life (Chen et al., 2008; Ashaolu, 2019). In this sense, the technological-food characterization and molecular taxonomy tools would be good alternatives to value these native bacteria strains from artisanal dairy products to improve traditional food technologies and develop new products.

The main food technological characteristics of LAB useful in dairy foods are (i) acidification activity to reduce the pH of milk in 6–24 h of incubation (Cogan et al., 1997; Beresford et al., 2001; Gonzalez-Gonzalez et al., 2019), (ii) proteolytic activity (Ardö et al., 2017; Meng et al., 2018) that hydrolyze casein into peptides and essential amino acids, improving flavor, texture, the production of volatile compounds and achieving better aroma (Biscola et al., 2016; Meng et al., 2018), (iii) lipolytic activity (El Soda et al., 1995; Silva et al., 2020) that biotransform free fatty acids to produce molecules, such as ethyl

hexanoate and ethyl butanoate (De Oliveira et al., 2021) and producing diacetyl that confers it a strong butter aroma (Wang S. et al., 2019; Kim et al., 2021). Hence, resistance to antibiotics is another property sought for microorganisms that do not have horizontal gene transfer against intestinal pathogenic or opportunistic bacteria (Miranda et al., 2021) and can be used as starters in functional foods (Shi et al., 2018).

In Peru, the main production of Swiss-type artisanal cheese is located in the regions of Cajamarca, La Libertad and Ancash, these products have great sensory acceptance by consumers (Barreto et al., 2010; Gálvez Antinori, 2020). However, the lactic microorganisms associated with this characteristic are scarcely studied, and even less their technological-food characterization. Therefore, lactic acid bacteria were isolated and characterized from Swiss-type cheese sold in Huaraz markets (Ancash, Peru). Finding LAB strains with technological-food benefits due to the reduction of acidification time, aroma improvement through their proteolytic and lipolytic activities, and resistance to some antibiotics that can support the recovery of the gastrointestinal microbiota.

2 Materials and methods

2.1 Sample

Three pieces of Swiss type cheese (Q1, Q2 and Q3) were purchased from a well-known cheese factory in Huaraz (Ancash, Peru). The samples were stored in aseptic conditions and transported to the laboratory for LAB isolation within 24 h collection.

2.2 Isolation and selection of lactic acid bacteria

All samples (Q1, Q2 and Q3) were washed with sterile water. Under sterile conditions, the outer layer (approx. 10 mm) was removed, cut, and three parts were randomly chosen to be adjusted to 25 g. Each sample was placed in a sterile Erlemmeyer flask with 225 mL of NaCl (0.85% w/v), and homogenized for 20 min at 700 rpm with a magnetic stirrer. Bacterial isolation was carried out by serial dilution, then 100 uL of 10^4 dilution of each sample was inoculated on MRS (Man, Rogosa and Sharpe) agar plates supplemented with nystatin (50 μg/mL) to prevent fungal and yeast growth and incubated at 37°C for 48 h under anaerobic conditions. Three replicates were prepared for each sample. Different colonies (color, size, border, etc.) were selected and consecutive streaks were made until a pure culture was obtained. Catalase activity and Gram staining were performed using standard protocols. Strains of catalase negative and Gram (+) bacteria were selected, main characteristics of LAB; to later be cryopreserved according to Ulloa-Muñoz et al. (2020).

2.3 Molecular identification

The taxonomic identification of the selected strains was carried out according to Ulloa-Muñoz et al. (2020). For this, the DNA was extracted from fresh bacterial culture pellets (MRS broth) with the GeneJET Genomic DNA Kit (Thermo Scientific, United States) following the manufacturer's guide. The 16S rDNA fragment was amplified by PCR

using the 27F/1492R primer set. The quality of the amplicons was checked by agarose electrophoresis (1%) and sequenced at Macrogen Inc. (Seoul, Korea) with the 518F/800R primer set. The forward and reverse sequences were edited, assembled and compared with the standard sequences of the Genbank,[1] which were downloaded to carry out the phylogenetic analysis that was executed in Mega V.7 using the Maximum Likelihood and Kimura-2-parameter methods with 1,000 bootstraps.

2.4 Inoculum preparation

The selected bacterial strains were grown anaerobically in MRS broth (5 mL) at 37°C for 24 h. The cultures were centrifuged at 2000 g x 5 min, the supernatant was discarded, the pellet was washed and resuspended in sterile NaCl (0.85%) and the optical density ($\lambda = 600$ nm) was adjusted to optical density at 600 nm (OD600): 0.8 equivalent to approximately 1×10^8 UFC/mL, according to Vera-Peña and Rodriguez-Rodriguez (2020) in a spectrophotometer UV–Vis (Thermo Scientific Genesys 150, United States).

2.5 Biosafety testing of LAB

2.5.1 Gelatinase activity

The essay was executed according to Sarasan et al. (2021) using MRS agar supplemented with gelatin (1%). Three sterile filter paper disks (5 mm) soaked with 5 μL of fresh bacterial inoculum (OD600 = 0.8) were placed. For the negative control, 5 μL of 0.8% saline solution was used. All samples were incubated at 37°C for 5 days. The gelatinase activity was revealed using a mercury (II) chloride solution (15 g of $HgCl_2$, 20 mL of HClcc and 100 mL of distilled water) added around the disks. Transparent halos reveal the production of gelatinases (Zafiro et al., 2007).

2.5.2 Hemolytic activity

The experiment was carried out according to the methodology proposed by Srinivash et al. (2023) using sterile TSA agar supplemented with 5% human blood under aseptic conditions. 3 sterile filter paper disks (5 mm) soaked with 5 μL of fresh bacterial inoculum (OD600 = 0.8) were placed. For the negative control, 5 μL of 0.8% saline solution. All samples were incubated at 37°C for 2 days. Green or light halos indicate hemolytic activity.

2.5.3 Antibacterial activity

One hundred μL of fresh LAB (OD600 = 0.8) was inoculated by incorporation into 20 mL of MRS agar, poured into a Petri dish and incubated at 37°C for 7 days under anaerobic conditions to induce the production of antimicrobial metabolites according to Tamariz-Angeles et al. (2023) with a few modification. *Staphylococcus aureus* ATCC25923, *Listeria monocytogenes* ATCC7644, *Escherichia coli* ATCC10536 and *Salmonella enterica sv* Typhimurium ATCC14028 were used for the antibacterial test. The strains were cultured in TSB broth (5 mL) at 37°C for 24 h and adjusted to an optical density of $OD_{620} = 0.08$ (Tamariz-Angeles et al., 2018), then 100 μL of the broth

was spread by extension on plates with Müller-Hinton Agar. Immediately, two BAL agar disks (5 mm) were cut and transfected onto the plates containing the pathogens. The plates were incubated at 37°C for 24 h. For the negative control, MRS agar disks (5 mm) without LAB were used. Clear halos indicated antibacterial activity and were measured in millimeters (mm).

2.5.4 Antibiotic sensitivity

The diffusion method was performed according to Singla et al. (2018) with some modifications. Standard disks of ampicillin (10 μg), erythromycin (15 μg), gentamicin (10 μg), penicillin (10 UI), rifampicin (30 μg), tetracycline (30 μg) and chloramphenicol (30 μg) were used. MRS agar plates were inoculated with 100 μL of fresh bacterial inoculum (DO600 = 0.8) and superficially spread. After 10 min, three disks per antibiotic were placed on the inoculated plates. They were incubated at 37°C for 24 h in anaerobic conditions. The diameters of the inhibition halos were measured with a digital vernier, and the results were expressed according to the Clinical and Laboratory Standards Institute (CLSI).

2.6 Technological characterization of LAB

2.6.1 Acidifying activity

The acidifying potential was determined according to Tsigkrimani et al. (2022) with some modifications. 100 mL of UHT (ultra-high temperature pasteurized) skimmed milk were inoculated with fresh cultures at a concentration of 1×10^8 UFC/mL, and incubated anaerobically at 37°C for 72 h. Three repetitions were performed for the strains and non-inoculated samples (negative control). The pH was measured with a potentiometer, the pH variation was obtained with formula (1), and the acidity was determined by volumetric titration with formula (2) according to Tyl and Sadler (2017). Both parameters were obtained at 0, 3, 6, 12, 24, 48 and 72 h of incubation under anaerobic conditions.

$$\Delta pH = pH_{initial} - pH_{final} \qquad (1)$$

$$\%\text{Acidity} = \left[\left(V_c \times N \times E_w \right) / \left(S_v \times 1000 \right) \right] \times 100 \qquad (2)$$

Where:
Vc: Volume of NaOH used in the titration.
N: Normality of the standard solution NaOH.
Ew: Lactic acid equivalent weight (90.08 g).
Sv: Sample volume.
1,000: Conversion factor (mg/g).

2.6.2 Proteolytic activity

The analysis was performed according to Petkova et al. (2021) with some modifications. The diffusion technique was used in wells (3 mm in diameter) in milk Agar (0.5% casein peptone, 0.25% yeast extract, 0.1% dextrose, 2.5% skimmed milk powder and 1.5% agar-agar) adjusted to pH 6.5 with HCl (3 N). Then, 50 μL of fresh bacterial inoculum was inoculated (OD600 = 0.8) in each well, and the plates were incubated at 37°C for 48 h under anaerobic conditions. Sterile MRS broth was used as a negative control and three replicates were

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1 http://www.ncbi.nlm.nih.gov/

prepared. The clear halo around a hole indicated positive proteolytic activity.

2.6.3 Lipolytic activity

The lipolytic activity was performed according to Dogan and Taskin (2021) and Carrim et al. (2006) with some modifications. The medium for the lipolytic screening contained: peptone (1.0%), $CaCl_2$ (0.01%) and agar-agar (1.5%). The pH was adjusted for 6.5 and supplemented with sterile Tween 20 (1.0%) under sterile conditions. Four sterile filter paper disks (5 mm) soaked with 5 μL of fresh bacterial inoculum were placed (OD600 = 0.8) in the lipolytic medium and incubated anaerobically at 37°C for 24 h. The opaque area around the disks was considered a positive result.

2.6.4 Diacetyl production

This parameter was carried out according to Margalho et al. (2020) with some modifications. A single colony was selected from a fresh culture plate, transferred to MRS broth (5 mL) and incubated anaerobically at 37°C for 24 h. Then, the culture was centrifuged at 4500 g x 15 min. Its precipitate was recovered and 0.01 g was resuspended in 10 mL of peptone solution (7.6%). This suspension (5 mL) was transferred to tubes with UHT skimmed milk (5 mL) and incubated anaerobically at 37°C for 24 h. One milliliter of culture was mixed with 250 μL of alpha-naphthol in 96° ethanol (1% w/v) and 250 μL of KOH (16% w/v), and incubated at 37°C for 10 min. An uninoculated sample was added as a control. Diacetyl production was considered positive by the presence of a red colored ring in the mixture. Its intensity was qualitatively quantified.

2.7 Application of selected LAB in cheese

2.7.1 Starter culture preparation

Lacticaseibacillus paracasei (CQ1 and KQ3) and *Lactiplantibacillus* sp. (QQ3) strains were selected due to the good potential they showed in previous tests. The selected strains were reactivated following the procedure previously described in section "2.4" with two washes to remove any remains of the broth. The strains were inoculated in 250 mL of UHT milk and incubated at 37°C for 24 h.

2.7.2 Production of traditional cheese from the Ancash region

The preparation of traditional cheese was carried out as shown in Figure 1. A volume of 20 L of raw milk with 6.6 pH and a density of 1.026 was acquired. The density was readjusted to a density of 1.030 [range recommended by NTP 202.001:2016 (NTP, 2016)] with powdered milk, pasteurized at 65°C for 30 min, $CaCl_2$ (0.025%) was added during pasteurization. It was then cooled to 38°C and divided into 5 L samples. The samples were inoculated with *L. paracasei* CQ1, *L. paracasei* KQ3 and *Lactiplantibacillus* sp. QQ3. A control without inoculum was produced. The samples were homogenized with a sterile steel paddle for 15 min. Rennet (enzyme) was added *Rhizomucor miehei*, CHR HANSEN, Denmark to each sample according to the manufacturer's instructions, always maintaining the temperature around 38°C, letting it rest for 45 min until the formation of curd which was later cut using a sterile stainless-steel knife, ensuring the size of 1 cm cubes. Later, it was drained using sterile meshes, the mass obtained was salted with 0.5% NaCl. This mass was placed in 500 g PVC molds to be molded

and pressed for 4 h. Finally, the cheese was removed from the mold and stored at 8°C for 8 days.

2.7.3 Sensory analysis

Sensory analysis was carried out according to Mauricio-Sandoval et al. (2023). Thirty-five panelists participated, regular cheese consumers, whose ages ranged from 18 to 74 years old. Each participant tasted four cheese samples of 10 g each, randomly coded. The sensorial attributes evaluated were flavor, odor, texture and general appearance, with scores from 1 to 5, where: (1) I dislike it a lot, (2) It displeases me, (3) It does not please me or it displeases me, (4) It does not please me it pleases me (5) It pleases me very much.

2.7.4 Physicochemical characteristics of traditional cheeses

Analyzes of moisture, ash, fat, protein, carbohydrates and pH of the cheeses were performed according to the procedures described in Barrios Renteria et al. (2022), according to the following methods:

Determination of moisture by difference in weight according to IDF-IDF 151:2005.

Determination of ash content according to A.O.A.C. 945.46:2002 method.

Determination of fat content according to IDF-IDF method 116A:1996.

Determination of protein according to the method of NTP 202.119:2014.

Determination of carbohydrates by the difference method of Collazos et al. (1993).

2.8 Statistical analysis

To determine the statistical significance of the values, the mean ± standard deviation (SD), ANOVA and Tukey's test were used (α =0.05), using the Statistical Package for the Social Sciences (SPSS) v.23.

3 Results and discussion

3.1 Isolation and phenotypic characterization of Swiss-type cheese bacteria

Fourteen strains were isolated from three pieces of artisanal cheese (Q1, Q2 and Q3) obtained in a traditional market in Huaraz (Peru). All isolates were Gram positive, while only 11 were catalase negative (Table 1). These strains had a bacillary shape consistent with Falsen et al. (1999) and Pundir et al. (2013), who described lactic acid bacteria as facultative anaerobic, Gram-positive, without endospore, catalase-negative and producing lactic acid as the main product.

3.2 Molecular taxonomic identification

Before the 20th century, the taxonomy of lactic acid bacteria was based on phenotypic characteristics (Klein et al., 1998), but currently the analysis of 16S rDNA has recorded a greater diversity of this

FIGURE 1
Ancash traditional cheese production flow diagram.
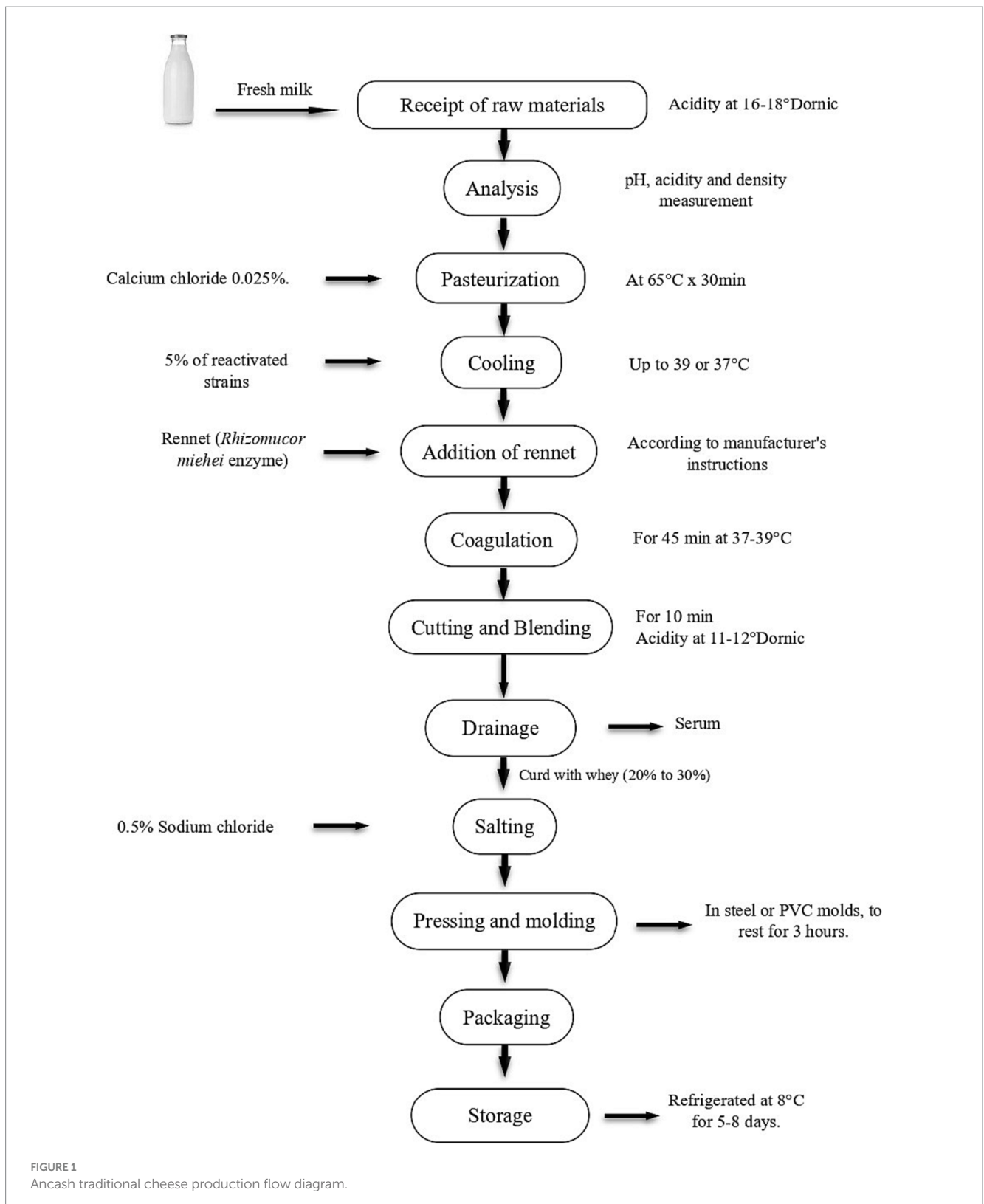
bacterial group (Tringe and Hugenholtz, 2008). At present, the genus *Lactobacillus* has been reclassified by Zheng et al. (2020) in 23 new genera, such as *Lacticaseibacillus*, *Lentilactobacillus* and *Lactiplantibacillus*, using a polyphasic method (phenotypic and genotypic).

64% of the bacterial strains isolated from the Swiss-type cheese analyzed belonged to *Lacticaseibacillus paracasei* (GQ2, EQ1, TQ1, YQ1, LQ2, CQ1, and KQ3), 27% to *Lentilactobacillus parabuchneri* (BQ2, OQ2, and RQ3) and 9% a *Lactiplantibacillus* sp. (QQ3) (Figure 2).

TABLE 1 Phenotypic characterization of the isolated bacteria.

Assays/ strains	TQ1	YQ1	QQ3	EQ1	HQ1	IQ1	LQ2	BQ2	GQ2	OQ2	KQ3	RQ3	CQ1	JQ3
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase activity	−	−	−	−	+	+	−	−	−	−	−	−	−	+

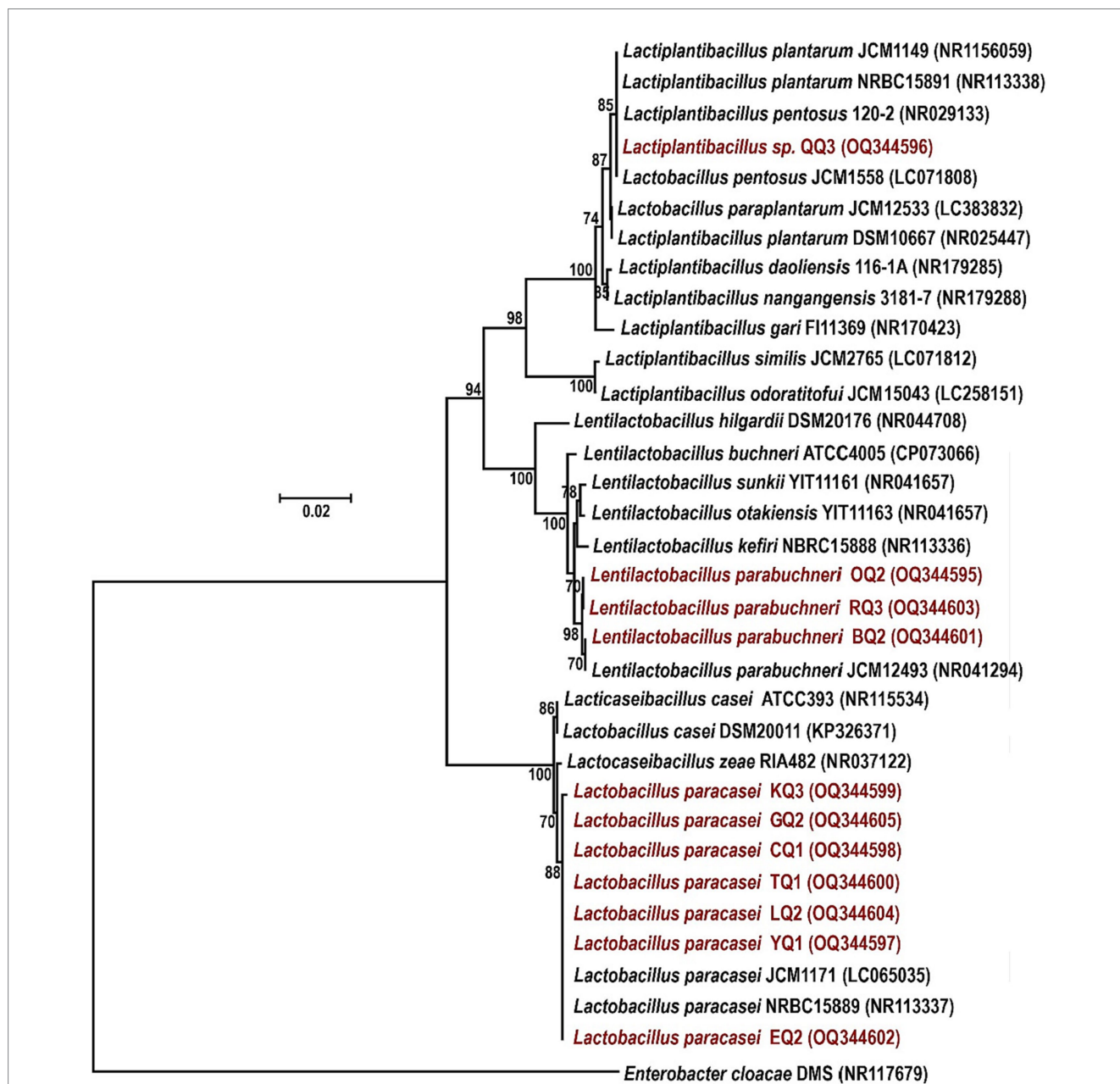HQ1, IQ1, and JQ3 were discarded due to the presence of catalase enzyme.

FIGURE 2
Phylogenetic analysis of lactic acid bacteria (LAB) from Swiss-type cheese from Ancash (Peru) using complete 16S rDNA. All phylogenetic trees were performed using the Likelihood and Kimura-2-parameter methods with 1,000 bootstraps. The letters in red are the isolated strains, in black the GenBank type strains, and in parentheses the GenBank access number.

Our findings were similar to those obtained by Meral Aktaş and Erdoğan (2022) who identified by 16S rDNA *Lacticaseibacillus paracasei* spp. *tolerans* L41 isolated from Turkish Beyaz cheese (white), classified as ripened cheese. Luiz et al. (2017) found *Lacticaseibacillus*, *Lentilactobacillus* and *Lactiplantibacillus* in Brazilian artisanal cheese, in addition to other bacterial strains belonging to the genera

Lactobacillus, *Enterococcus*, *Lactococcus* and *Pediococcus*. However, Mohammed and Çon (2021) in Turkish white cheese isolated bacteria of the genera *Enterococcus*, *Lactiplantibacillus* and *Loigolactobacillus*, but no *Lacticaseibacillus* and *Lentilactobacillus* were found. The isolation of the diversity of bacteria is related to the medium used (Domingos-Lopes et al., 2017). Therefore, some factors that determine this diversity could be the variety of cheese, the manufacturing process, coagulation, the types of whey drainage (Afshari et al., 2020), milk pasteurization, addition of lactic ferments (Zoumpopoulou et al., 2020), place of provenance of the cheese sample, species and breed of mammal from which the milk comes (Tulini et al., 2016; Islam et al., 2021). In this investigation, the high percentage of species associated with the genus *Lactobacillus* may be due to the selective property of the MRS medium.

Lacticaseibacillus strains belong to the homofermentative group, produce lactic acid from glucose and grow between 10 and 45°C. Some species are used as starter cultures or probiotics (Zheng et al., 2020). Other species have no effect on initial acidification, but play an important role in ripening (Levante et al., 2020b). Barzideh et al. (2022) found an abundance of 15–90% of the species in cheeses aged 13–32 months. In addition, this genus had been identified and used in different types of cheeses such as Parmigiano Reggiano (Levante et al., 2020a), Pecorino di Farindola (Aprea et al., 2021), Turkish Beyaz (Meral Aktaş and Erdoğan, 2022), traditional Iranian (Afshari et al., 2022), Cheddar (Leeuwendaal et al., 2022), DOP Greek Galotyri (Samelis and Kakouri, 2022). Similarly, the genus *Lactiplantibacillus* belongs to the group of homofermentatives. They can metabolize different phenolic acids by various metabolic pathways, also characterized as starter cultures or probiotics and have been isolated from fermented products and vertebrate intestinal tracts (Zheng et al., 2020). This genus has been identified and used in Greek Feta cheese (Papadopoulou et al., 2018), Cheddar (Wang J. et al., 2019), Turkish white cheese (Mohammed and Çon, 2021), Brazilian artisanal cheese (Luiz et al., 2017). Unlike the genera *Lacticaseibacillus* and *Lactiplantibacillus*, bacteria of the genus *Lentilactobacillus* are heterofermentative and slow growing at temperatures between 15 and 45°C (Zheng et al., 2020), this genus has been cited as a microbiota of Greek Galotyri DOP cheese (Samelis and Kakouri, 2022), DOP Azeitão cheese (Cardinali et al., 2021), "Serrano" cheese (Margalho et al., 2021). *Lentilactobacillus parabuchneri FAM 21835,* a non-histamine strain, isolated from Swiss Tilsit type cheese, influences ripening and is responsible for the formation of the cheese eye, flavor, texture, and the production of ornithine, glutamic acid, γ-aminobutyric acid and histidine (Fröhlich-Wyder et al., 2015), and could present an inhibitory effect against mycelial fungi (Liang et al., 2022).

The identification of bacterial strains of the genera *Lacticaseibacillus*, *Lactiplantibacillus* and *Lentilactobacillus* of Swiss-type cheeses from Huaraz allowed to know their microbial diversity and the role of these strains in this type of cheese, in addition, according to the technological characteristics, each bacterial strain could be proposed as a candidate to improve or produce new products. Mainly, in the elaboration of the Swiss type cheese, it is used: pasteurized cow's milk, lactic culture (R-707 = *Lactococcus lactis* subsp. *lactis* bio*var diacetylactis*), rennet, calcium chloride, preservative and salt (Bekele et al., 2019; Guevara Cruzado and Reyes Flores, 2019), but during its ripening or semi-ripening, a natural secondary microflora grows that is responsible for its sensory acceptability (Blaya et al., 2018). In addition, species of *Lentilactobacillus* were detected in raw milk (Carafa et al., 2019). The Ancash Swiss-type artisanal cheese is

mainly made with pasteurized milk without the addition of microorganisms, but three kinds of LAB were found. According to Pundir et al. (2013), its presence in this type of products may be due to contamination after milk pasteurization because there are microorganisms in the air, the equipment or the ingredients involved in its preparation; also some LAB may be resistant to pasteurization or transfer during product storage (Stefanovic et al., 2018). Furthermore, *Lactiplantibacillus* species are used as starter cultures in various fermented foods to develop organoleptic and probiotic characteristics (Metrouh et al., 2022).

3.3 Biosafety testing of LAB

3.3.1 Gelatinase and hemolytic activity

The selected LAB did not show gelatinase activity, likewise, it was negative for the α-hemolysis test and β-hemolysis. Cizeikiene and Jagelaviciute (2021) reported that the genders *Lactobacillus* and *Bifidobacterium* did not present gelatinase activity. Srinivash et al. (2023) isolated four LAB (*L. delbruecki*, *L. hircilactis*, *L. jhonsonii* and *L. lechmannii*) which also did not show hemolytic activity, as reported for *L. plantarum* UBLP40 (Ahire et al., 2021). Both assays with negative results are requirements for the selection of safe probiotic strains (Ahire et al., 2021; Srinivash et al., 2023).

3.4 Antibacterial activity

Overuse of antibiotics makes pathogenic bacteria more resistant, causing the search for new LAB strains that can inhibit these pathogens (Miao et al., 2016) and be used in the production of new foods, especially cheese (Abosereh et al., 2016) and extend their shelf life (Silva et al., 2023).

The selected LAB showed inhibitory action on pathogenic bacteria (Figure 3). The strains that presented the best results were *L. paracasei* (KQ3) which inhibited *S. aureus* (3 mm) and *E. coli* (4.5 mm), *L. parabuchneri* (BQ2) inhibited *L. monocytogenes* (8 mm) (Supplementary Figure S1) and *S. enterica sv* Typhimurium (5.5 mm) and *L. paracasei* (GQ2) inhibited *E. coli* (4.5 mm). The inhibitory action of species of the *Lactobacillus* genus was reported by Tambekar and Bhutada (2010) and Shehata et al. (2022).

The antibacterial activity of LAB is possibly related to the production of organic acids, mainly lactic acid (Zhang et al., 2020), and others such as acetic acid, citric and tartaric acids (Zalán et al., 2010) or bacteriocins (Zhang et al., 2020) which inhibit pathogenic microbes and generates minimal resistance (Miao et al., 2016). It can be related to the production of ethanol, diacetyl or substances similar to antibiotics (Bintsis, 2017). The metabolites synthesized by the LAB can prevent the colonization of pathogens in the body by competing for adhesive and nutrient receptors (Barzegar et al., 2021), using preventive and therapeutic purposes to control enteric infections (Tambekar and Bhutada, 2010).

3.5 Antibiotic sensitivity

Genes of resistance to transmissible or transferable antibiotics of the *Lactobacillus* genera are of increasing concern (Lucumi-Banguero et al., 2021) for human and animal health (Reuben et al., 2020),

because some probiotic strains could transfer antibiotic resistance genes to pathogenic bacteria causing problems for their consumers (Mangia et al., 2019; Wang K. et al., 2019).

The antibiotic susceptibility results performed by the diffusion method are found in Table 2. It was observed that all LAB strains (100%) were sensitive to rifampicin (30 μg), erythromycin (15 μg), penicillin (10 IU) and gentamicin (10 μg); 64% (NQ1, EQ1, LQ2, BQ2, GQ2, KQ3 and CQ1) were sensitive to tetracycline (30 μg), 18% (QQ3 and LQ2) to ampicillin (10 μg) and 9% (QQ3) to chloramphenicol (30 μg) and vancomycin (30 μg) (Supplementary Figure S2). Similar to this study, *L. paracasei* FS103 isolated from sheep cheese showed sensitivity to penicillin, erythromycin, and tetracycline, among others (Mangia et al., 2019). Meral Aktaş and Erdoğan (2022) and Zoumpopoulou et al. (2017) found that the strains *L. paracasei* subsp.

tolerans L41, ACA-DC 177, and ACA-DC 196 isolated from Turkish Beyaz (white) and Greek Kasseri cheese that showed sensitivity to chloramphenicol but resistance to gentamicin, these results were similar to other strains reported by Metrouh et al. (2022). *L. paracasei* subsp. tolerans isolated from traditional Ethiopian fermented food presented sensitivity to tetracycline, ampicillin and erythromycin (Mulaw et al., 2019).

Regarding antibiotic resistance of *Lentilactobacillus* sp., Nawaz et al. (2011) isolated 12 *L. parabuchneri* of fermented vegetables, and seven strains were resistant to gentamicin and two strains to chloramphenicol. This demonstrated that not all LAB of the same species have resistance to the same antibiotics and vary by antibiotic concentration (Barzegar et al., 2021; Randazzo et al., 2021) or by intrinsic genetics of *Lactobacillus* strains that are resistant to aminoglycosides or glycopeptides such as gentamicin and vancomicin (Akpınar Kankaya and Tuncer, 2020; Jomehzadeh et al., 2020). Therefore, it is suggested that LAB resistance to antibiotics may be convenient to be studied whether this characteristic is associated with mobile genetic elements, such as plasmids or transposons, to determine the possibility of horizontal transfer to pathogenic bacteria (Kang et al., 2020; Parlindungan et al., 2021) or by conjugation of LAB isolated from raw milk, artisanal and non-artisanal cheese resistant to penicillin, erythromycin, tetracycline and rifampicin (Russo et al., 2018; Margalho et al., 2020; Výrostková et al., 2020). There is a particular case of resistance to chloramphenicol associated with the chloramphenicol acetyltransferase (cat) gene located in *Lactobacillus* plasmids that is not common in bacteria of this genus (Leeuwendaal et al., 2021; Sharma et al., 2021). Furthermore, the cat gene is not transferred between food and non-food bacteria (Abriouel et al., 2015).

Some authors consider that the antibiotic resistance of LAB could be an opportunity for the food industry because these strains can help to reposit the intestinal microbiota during the administration of antibiotics to control diarrheal problems (Wang K. et al., 2019; Huang et al., 2021; Islam et al., 2021), but it is a desirable that the gene involved cannot be transmitted to pathogenic bacteria (Kamarinou et al., 2022). In addition, antibiotic resistance in LAB could be due to the indiscriminate use of antibiotics in animals, mainly in this context,
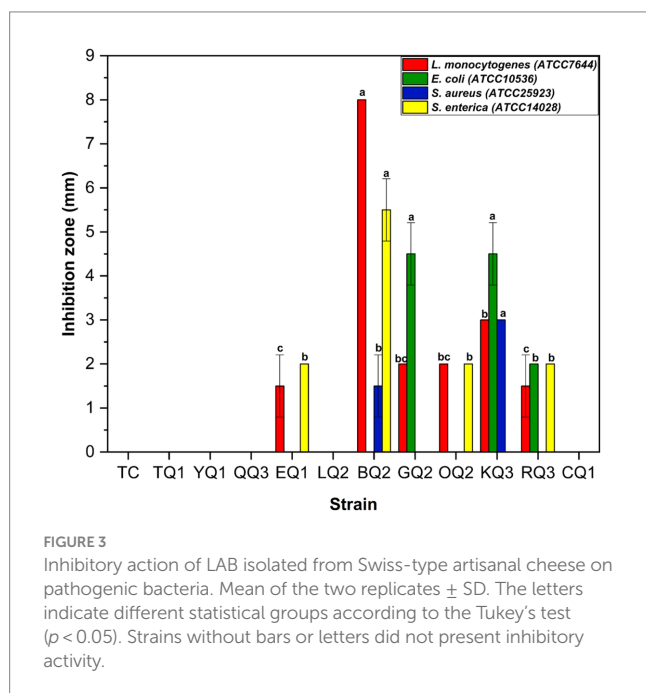

FIGURE 3
Inhibitory action of LAB isolated from Swiss-type artisanal cheese on pathogenic bacteria. Mean of the two replicates ± SD. The letters indicate different statistical groups according to the Tukey's test ($p < 0.05$). Strains without bars or letters did not present inhibitory activity.

TABLE 2 Antibiotic susceptibility test by the diffusion method of LAB isolated from Swiss-type artisanal cheese.

Isolated strain	RIF	TE	AM	GE	E	PE	CHL	VA
TQ1	33 (S)	20 (I)	20 (I)	1 (R)	31 (S)	31 (S)	2 (R)	6 (R)
YQ1	29 (S)	21 (S)	10 (R)	5 (R)	29 (S)	24 (S)	0 (R)	5 (R)
QQ3	25 (S)	10 (R)	27 (S)	13 (R)	30 (S)	25 (S)	22 (S)	16 (I)
EQ1	33 (S)	30 (S)	10 (R)	2 (R)	28 (S)	26 (S)	4 (R)	9 (R)
LQ2	34 (S)	26 (S)	21 (S)	3 (R)	30 (S)	34 (S)	1 (R)	10 (R)
BQ2	29 (S)	25 (S)	16 (I)	4 (R)	27 (S)	22 (S)	1 (R)	4 (R)
GQ2	28 (S)	25 (S)	11 (R)	2 (R)	28 (S)	25 (S)	2 (R)	8 (R)
OQ2	37 (S)	10 (R)	10 (R)	14 (R)	35 (S)	29 (S)	6 (R)	2 (R)
KQ3	32 (S)	22 (S)	9 (R)	1 (R)	26 (S)	24 (S)	1 (R)	5 (R)
RQ3	34 (S)	8 (R)	9 (R)	12 (R)	33 (S)	28 (S)	8 (R)	9 (R)
CQ1	31 (S)	25 (S)	15 (R)	3 (R)	31 (S)	31 (S)	3 (R)	10 (R)

*RIF: Rifampicin (30 μg); TE: Tetracycline (30 μg); AM: Ampicillin (10 μg); GE: Gentamicin (10 μg); E: Erithromycin (15 μg); PE: Penicillin (10 UI); CHL: Chloramphenicol (30 μg); VA: Vancomicin (30 μg).

**(R): Resistant (≤15 mm); (I): Intermediate (16-20 mm); (S): Sensible (≥21 mm).

this trait in LAB isolated from dairy products marketed in rural areas, where antimicrobial agents are rarely used, could be associated with intrinsic resistance to non-transferable genes (Kakelar et al., 2019), and strains belonging to the *Lactobacillus* group could have natural antibiotic resistance genes associated with their chromosome (Kamarinou et al., 2022). Therefore, the resistance to antibiotics shown by the bacteria isolated in this research could be the focus of future studies.

3.6 Acidifying activity of LAB

The lactic acid produced by LAB is necessary for the preparation of cheese and fermented foods, it increases acidity and lowers pH, improving flavor and rheological properties and eliminates pathogenic or spoilage bacteria (Campagnollo et al., 2018; Barzegar et al., 2021). It could also enhance biological activity through the production of bacteriocins and exopolysaccharides (Belleggia et al., 2022).

Figure 4 shows the pH of the inoculated milk at different times, where the initial pH of the milk was 6.6. The pH reduction was weak after 6 h in all LAB strains tested, however, *L. paracasei* KQ3 strain reduced the milk pH to 5.2 in 12 h, and this strain and *L. paracasei* CQ1 showed higher pH reduction near 4.0 (Supplementary Table S1). Moreover, the △pH in all the samples inoculated with LAB isolated from Swiss type cheese was 0.43–2.47 and 1.90–3.10 at 24 and 72 h, respectively, in which *L. paracasei* (KQ3, CQ1, TQ1, and EQ1) and *Lactiplantibacillus* sp. (QQ3) can be considered as potential acidifying strains. A similar result was found in *L. paracasei* subsp. *tolerans* HUM15, isolated from camel milk, which could decrease the pH to 4.42 after 72 h of incubation (Hawaz et al., 2016). On the other hand, Pisano et al. (2022) reported that *L. plantarum* 4/16898 from sheep's milk cheese presented 2.22 △pH at 24 h, value less than *Lactiplantibacillus* sp. QQ3.

According to the classification of Albayrak and Duran (2021), none of the strains in this research could be considered good acidifiers because they defined that good acidifying bacteria reduce the pH of milk from 6.6 to 5.3 or increase the acidity ≥0.25 g of lactic acid per 100 mL after 6 h of incubation. Nevertheless, Mohammed and Çon (2021) classified bacteria as strong acidifiers when ΔpH 24 h ≥ 1.61 or ΔpH 72 h ≥ 1.63 and weak acidifiers with ΔpH 24 h ≤ 1.11. *L. paracasei* TQ1, EQ1, CQ1 and KQ3, and *Lactiplantibacillus* sp. QQ3 exhibited △pH 24 h between 1.8–2.47. These bacterial strains are strong acidifiers in contrast to *L. parabuchneri* BQ2, OQ2, RQ3, and *L. paracasei* YQ1, LQ2, GQ2 which showed a weak acidification capacity following the classification of Mohammed and Çon (2021).

The pH is inversely proportional to acidity (Fauziah et al., 2020). Figure 4 shows that the initial acidity percentage of the milk was 0.16 and that *L. paracasei* TQ1, EQ1, CQ, KQ3 and *Lactiplantibacillus* sp. QQ3 increased this acidity to 0.38–0.71 (24 h) and 0.77–1.28 (72 h), considered high acidification values. Meral Aktaş and Erdoğan (2022) isolated *L. paracasei* subsp. *tolerans* L41 of Turkish Beyaz (white) cheese, which barely increased the acidity of the milk close to 0.20 with pH > 6.0 after 24 h. Furthermore, a strain of *L. parabuchneri* involved in the production of PDO Italian Bitto cheese showed a slow acidification process (Morandi et al., 2011) similar to *L. parabuchneri* BQ2, OQ2, RQ3 isolated in this investigation (Supplementary Table S1).

Although it is true that bacteria of the genus *Lactobacillus* metabolize lactose slowly (Dagdemir and Ozdemir, 2008), it was

observed that after 24 h of incubation, some strains increased the acidity of the milk equal to or more than the acidifying bacteria (Ribeiro et al., 2021). According to González-González et al. (2022), LAB should reduce the pH < 4.7 after 24 h of incubation, but Monteagudo-Mera et al. (2011) mention that the artisanal production of cheese only requires bacterial strains with the capacity to reduce the pH of milk to 5.10 after 24 h of incubation. In agreement, *L. paracasei* TQ1, EQ1, CQ1, KQ3 and *Lactiplantibacillus* sp. QQ3 isolated in this research, show an interesting potential to be used as acidifying bacteria in dairy products.

3.7 Proteolytic activity

The proteolytic activity of LAB improves the sensory characteristics of fermented foods and plays an important role in the growth of other fermented bacteria (Islam et al., 2021). During the proteolytic event, high weight and complex proteins (casein micelles) are degraded to low molecular weight proteins and/or amino acids (Bezerra et al., 2016; Ardö et al., 2017), aromatic compounds such as aldehydes, alcohols, ketones, esters, etc. are also produced and released (De Souza and Silva Dias, 2017; Meng et al., 2018). In this investigation, all isolates showed proteolytic activity (Figure 5) in which *L. paracasei* CQ1, *Lactiplantibacillus* sp. QQ3, and *L. paracasei* KQ3 exhibited better degradation halos of 20.8, 16.0 and 11.5 mm, respectively (Table 3; Supplementary Figure S3).

Likewise, *L. paracasei* subsp. *tolerans* HUM15 isolated from camel milk showed proteolytic activity with a casein degradation halo of 15 mm (Hawaz et al., 2016), on the other hand, *L. paracasei* subsp. *tolerans* L41 isolated from Turkish Beyaz (white) cheese did not show proteolytic activity (Meral Aktaş and Erdoğan, 2022). Strains of *Lentilactobacillus*, *L. kefiri* and *L. buchneri*, isolated from cheese, showed a high proteolytic activity (Margalho et al., 2021; Akbulut et al., 2022). As for the species *Lactiplantibacillus* SJ14 isolated from Argelia cheese showed a 13 mm proteolytic halo on milk agar plates (Metrouh et al., 2022).

In general, the proteolytic activity of LABs is considered a desirable feature in the manufacture of fermented food (Panebianco et al., 2021), but it also contributes to the health sector, because they are used to prevent allergies in children (0–3 years) with poor digestion of the proteins of some dairy products (Meng et al., 2018; Islam et al., 2021). Thus, the LAB isolated in this research could be used in the manufacture of dairy products with better digestibility due to their proteolytic activity.

3.8 Lipolytic activity

Lipolytic enzymes (lipases) isolated from microorganisms are widely required in the food industry (Chandra et al., 2020) due to their stability, low production cost and easy access compared to plant or animal lipases (Tanasupawat et al., 2015). This enzyme is mainly used in dairy, meat, pickle, and bakery products to improve flavor (Salgado et al., 2022). In this investigation, all isolated LAB showed lipolytic activity, while *Lactiplantibacillus* sp. QQ3, *Lacticaseibacillus paracasei* CQ1, and LQ2, and *Lentilactobacillus parabuchneri* BQ2 showed higher degradative halos (30.8–36.3 mm) (Table 3; Supplementary Figure S3), in contrast to the other strains showed degradation halos below 27 mm (Figure 6).
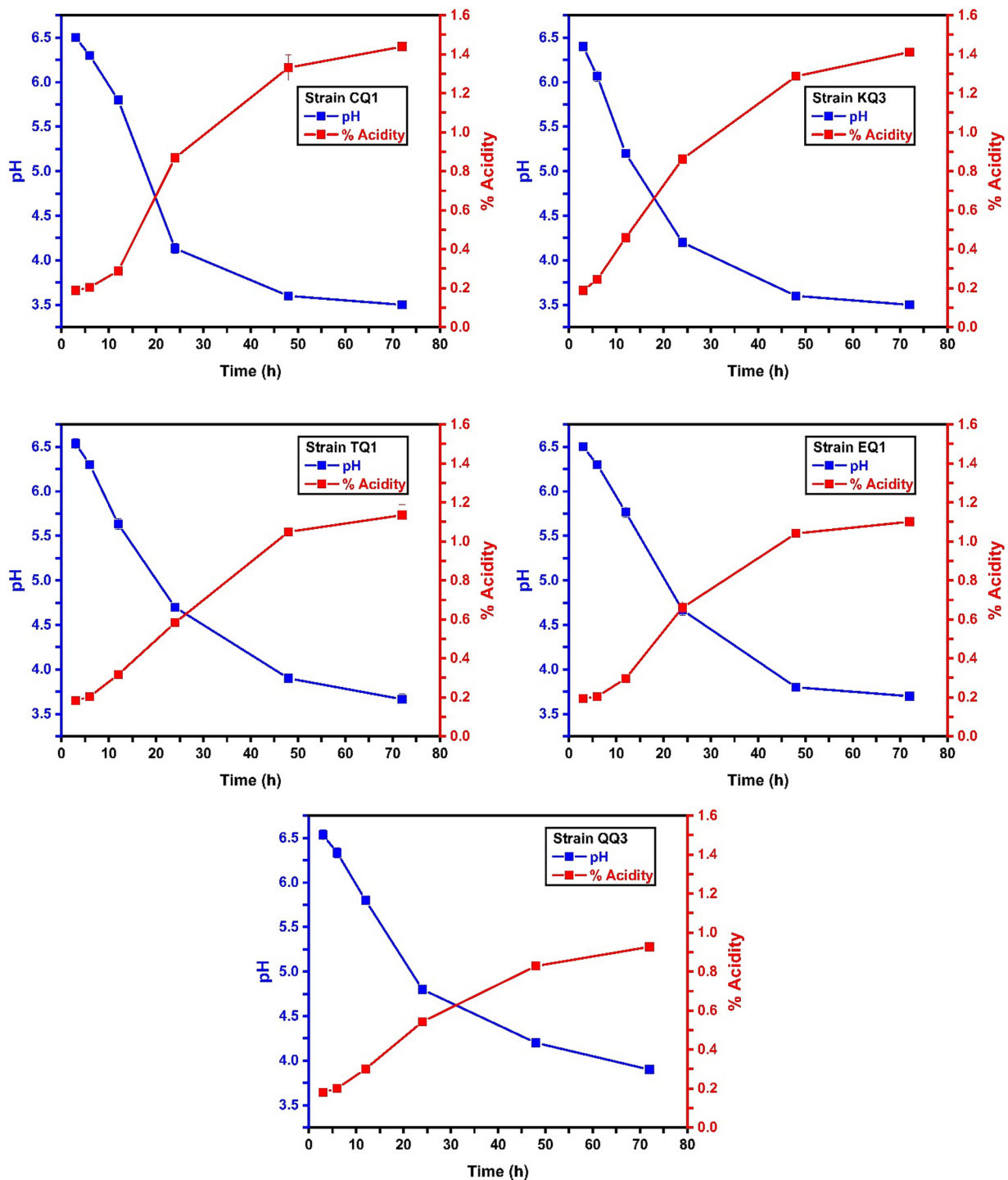
FIGURE 4
pH and Acidity versus time. The figure shows the behavior of the pH and acidity of the strains (CQ1, EQ1, GQ2, KQ3, QQ3 and TQ1) inoculated in milk, versus their incubation time (3, 6, 12, 24, 48 and 72 h).

Metrouh et al. (2022) reported a strain of *Lactiplantibacillus plantarum* SJ14 isolated from Argelia cheese and found lipolytic activity in culture media containing olive oil (3%) and Tween 80 (5%) with degradation halos of 14.5 mm and 10 mm, respectively. These values were lower than the halo of degradation of *Lactiplantibacillus* sp. QQ3 (36.3 mm) isolated from Ancash Swiss type cheese. In addition, some studies reported that strains of *Lacticaseibacillus casei* and *L. paracasei* isolates from Iranian raw cow's milk cheese, artisan

cheese and goat cheese did not show lipolytic activity (Câmara et al., 2019; Barzegar et al., 2021). The absence of lipolysis could be due to the culture medium used (Tributyrin agar). Carrazco-Palafox et al. (2018) mentioned that the commercial medium sometimes lacks calcium or magnesium ions that allow to intensify and stabilize giving greater visibility to the halo of degradation. However, Araújo-Rodrigues et al. (2021) found that *L. paracasei* isolated from PDO Serpa cheese showed a high lipolytic activity assayed on tributyrin
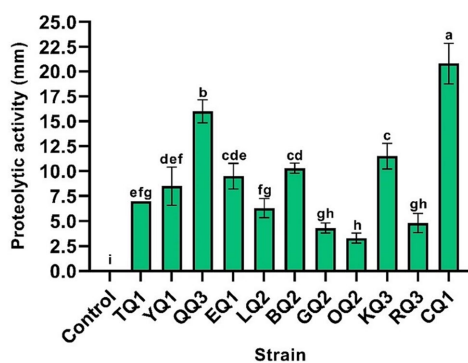
FIGURE 5
Proteolytic activity of LAB isolated from Ancash Swiss cheese. It is expressed as halos of milk protein degradation after incubation on agar plates. Mean of four replicates ± SD. The letters indicate different statistical groups according to the Tukey's Test ($p < 0.05$).

TABLE 3 Proteolytic and lipolytic activity and diacetyl production of Swiss type cheese.

Strain	Proteolytic activity (mm)	Lipolytic activity (mm)	Diacetyl production
Control	0.0 ± 0.0[i]	0.0 ± 0.0[g]	−
TQ1	7.0 ± 0.0[efg]	16.0 ± 2.7[f]	+
YQ1	8.5 ± 1.9[def]	22.0 ± 2.9[def]	−
QQ3	16.0 ± 1.2[b]	36.3 ± 5.0[a]	++
EQ1	9.5 ± 1.3[cde]	31.5 ± 3.1[ab]	−
LQ2	6.3 ± 1.0[fg]	30.8 ± 2.9[abc]	−
BQ2	10.3 ± 0.6[cd]	27.0 ± 2.0[bcd]	−
GQ2	4.3 ± 0.6[gh]	23.5 ± 1.9[de]	−
OQ2	3.3 ± 0.6[h]	24.0 ± 2.7[cde]	−
KQ3	11.5 ± 1.3[c]	22.8 ± 0.5[def]	−
RQ3	4.8 ± 1.0[gh]	18.3 ± 1.3[ef]	−
CQ1	20.8 ± 2.1[a]	35.5 ± 4.2[a]	−

Values are means from three replicates ± SD. Letters are statistic groups according to Tukey test ($p < 0.05$).

agar, so this activity may be related to the genetic component of this species.

Lentilactobacillus strains isolated in the present study have lipolytic activity while *Lentilactobacillus otakiensis* and *L. buchneri* strains isolated from pig feces and artisanal Pico cheese did not show such activity (Câmara et al., 2019; Marchwińska and Gwiazdowska, 2022). These results may be related to the weak production of lipase in some *Lactobacillus* bacteria (Ramakrishnan et al., 2019) including other genera later reclassified by Zheng et al. (2020). Then, all LAB isolated in the present investigation could be used effectively in the food industries.

3.9 Diacetyl production

Diacetyl is a volatile aromatic compound (Kim et al., 2021) highly required in dairy food and it is found mainly in cheese, yogurt, butter, buttermilk, etc (Chen et al., 2017; Domingos-Lopes et al., 2017).

Lactobacillus species are considered main producers of diacetyl, but currently, strains belonging to the genera *Streptococcus*, *Leuconostoc*, *Streptococcus*, *Pediococcus* and *Oenococcus* also show this capacity (Clark and Winter, 2015; Wang S. et al., 2019). Its synthesis is carried out by citrate lyase and α-acetolactate synthase that degrade milk citrate to α-acetolactate (an unstable molecule), which is decarboxylated as diacetyl at low pH (Hugenholtz et al., 2000; De Souza and Silva Dias, 2017).

The diacetyl produced by LAB improves the sensory acceptability of fermented products such as ripened cheese (Khattab et al., 2019), providing end products with buttery, creamy, sweet and milky flavors (Tian et al., 2020). In this study, it was found that *Lactiplantibacillus* sp. QQ3 showed better diacetyl production, and the fermented milk reacted immediately to the specific test, followed by *L. paracasei* TQ1, which showed a slow positive reaction after 10 min (Table 3). Metrouh et al. (2022) found out that *L. plantarum* SJ14, isolated from Algerian cheese, moderately produced diacetyl. Eight strains of *L. paracasei* out of 16 strains isolated from goat milk produced diacetyl (Meng et al., 2018), five strains of *L. paracasei* (PN 7, PN 59, PN 76, PN 105 and PN 145) of six strains isolated from PDO Provola dei Nebrodi Aquiartisanal cheese also showed the ability to produce this compound (Randazzo et al., 2021). Regardless of whether LAB are taxonomically classified by similar characteristics, strains of the same species may have different behavior or metabolic capacity to produce various types of volatile aromatic compounds or other metabolites (Agostini et al., 2018), these characteristics depend on the intrinsic phylogenetic properties of each strain (Carvalho et al., 2014; Ouattara et al., 2017).

Another application of diacetyl in food is its antimicrobial activity against pathogens (Meng et al., 2018; Wang S. et al., 2019) because it crosses the bacterial membrane and prevents the assimilation of arginine that blocks the enzymes involved in microbial development and growth (de Souza and Silva Dias, 2017). Thus, *L. paracasei* TQ1 and *Lactiplantibacillus* sp. QQ3 isolated from Ancash cheese could be used in fermented products to improve aroma, and perhaps extend shelf life if the diacetyl produced inhibits the growth of pathogenic or spoilage bacteria.

3.10 Sensory analysis

The sensory analysis was carried out on the first and eighth days of ripening (Figure 7). On the first day of ripening, both the cheeses inoculated with the LAB strains and the control without inoculum did not show statistically significant differences ($p < 0.05$; Supplementary Table S2). However, after 8 days of ripening, the cheese inoculated with LAB presented better attributes of flavor, aroma, texture and general appearance compared to the first day and the uninoculated control. This demonstrates the fermentative capacity in the food matrix of the LAB (Figure 7) improved the quality of the cheese. Awad et al. (2007) reported that the low acceptability of cheese is due to short fermentation periods and the lack of bacterial consortia.

Cheese made with the CQ1 strain received the highest scores for flavor (4.4 ± 0.60), texture (4.34 ± 0.59) and general appearance (4.31 ± 0.76). For aroma, the strains CQ1 (4.43 ± 0.50) and QQ3 (4.49 ± 0.61) obtained the highest score (Supplementary Table S3). Therefore, the acceptance of cheese inoculated with strains CQ1 and KQ3 may be related to the level of proteolysis and lipolysis exerted by these strains (Figures 5, 6). Coelho et al. (2022) and Araújo-Rodrigues et al. (2023) mention that amino acids and fatty acids released during
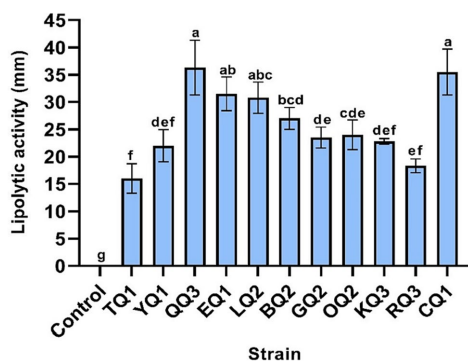
FIGURE 6
Lipolytic activity of LAB isolated from Ancash Swiss cheese. It is expressed as the diameter of the degradation halos after incubation on agar plates supplemented with Tween-20. Mean of four replicates ± SD. The letters indicate different statistical groups according to the Tukey test ($p < 0.05$).

proteolysis and lipolysis play an active role as substrates in several secondary reactions that drive the flavor and texture of cheese. The good acceptance of the aroma of cheese inoculated with the QQ3 strain could be related to the production of diacetyl (Supplementary Table S2), which is one of the main compounds responsible for the flavor and aroma in dairy products such as cheese and yogurt (Hernandez-Valdes et al., 2020). Likewise, Jo et al. (2018) demonstrated the contribution of diacetyl to the buttery flavor in young Gouda cheese.

In general, the strains used in cheese production seem to be promising in improving the sensory properties of cheese (Supplementary Figure S4). However, sensory acceptability could be further improved by increasing the maturation time and using these LAB in consortia in which each bacteria could contribute specific characteristics to the final product. Awad et al. (2007) mentioned that cheese made with bacterial consortia made up of *L. paracasei* subsp. *paracasei* added along with cultures such as *L. lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Streptococcus thermophilus* and *Lactobacillus helveticus* are sensory more acceptable.
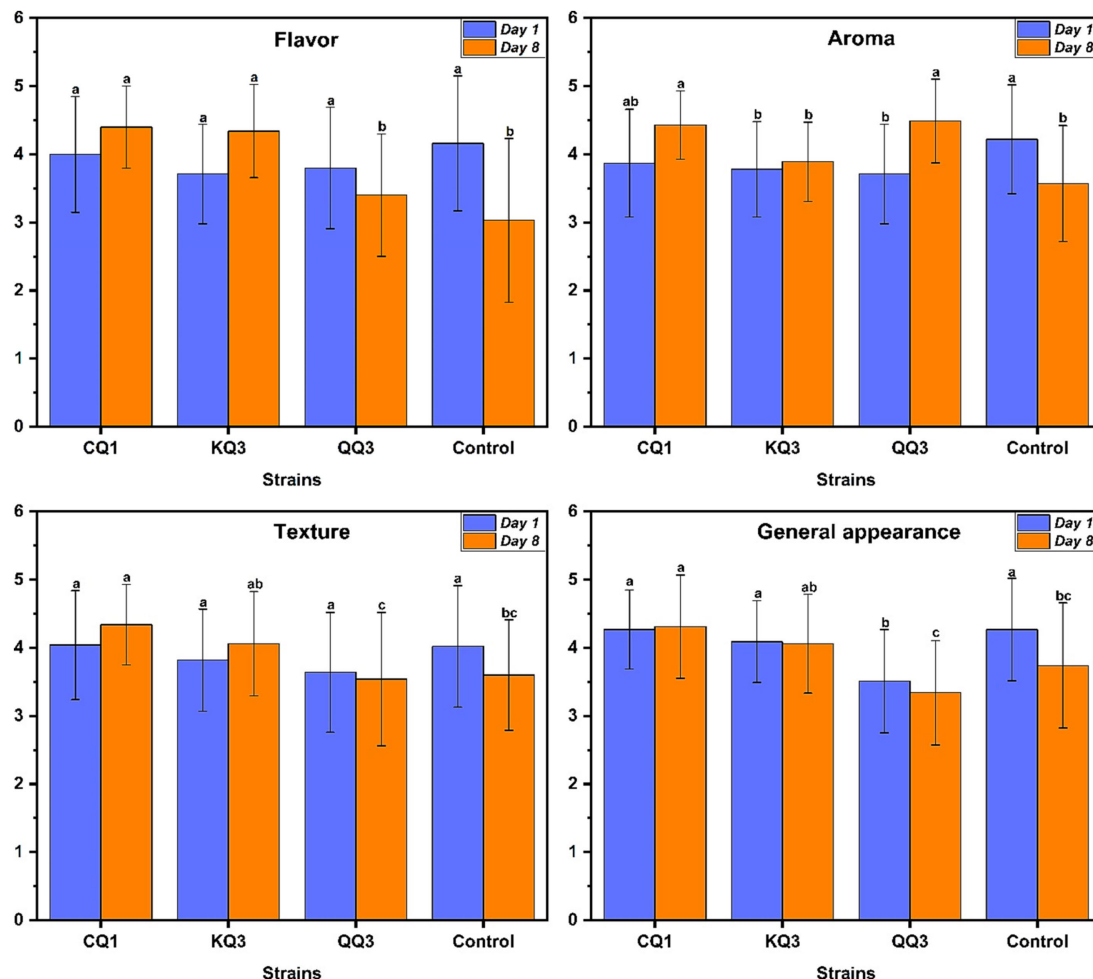

FIGURE 7
Effect of strains on the sensory properties of cheese. The figure shows the effect of the three selected strains (CQ1: *L. paracasei*, KQ3: *L. paracasei* and QQ3: *Lactiplantibacillus* sp.) in the sensory properties of the cheese (flavor, smell, texture and appearance in general) on the first and eighth days of fermentation. The letters indicate different statistical groups according to Tukey's test ($p<0.05$).

TABLE 4 Physicochemical characteristics of cheese without inoculum and cheese made with *L. paracasei* CQ1.

Physicochemical characteristics	Control	CQ1
Moisture (g/100 g)	44.29	34
Ash (g/100 g)	4.64	6.2
Fat (g/100 g)	23.55	32.05
Protein (g/100 g)	26.12	25.1
Carbohydrates (g/100 g)	1.4	2.65
pH	6.1	4.8

3.11 Physicochemical characteristics of traditional cheeses

Table 4 shows the proximal composition of the cheese made with *L. paracasei* CQ1 (sensorially better rated cheese) and the control (without inoculum) at 8 days of ripening, these results are within the expected parameters. The moisture content of cheese made with *L. paracasei* CQ1 was recorded at 34%, lower than the control (44.29%). This suggests that the reduction of pH in cheese with *L. paracasei* CQ1 is due to the production of lactic acid by this strain, which in turn facilitates gel syneresis and whey expulsion, as mentioned in previous research (Doolan et al., 2014). As a consequence of this syneresis, ash, fat, protein and carbohydrate values increased relative to the total sample mass. Values close to these results have been reported at 7 days of ripening in Graviera cheese, when strains of *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Streptococcus thermophilus* and *Lactobacillus helveticus* were added (Ioannidou et al., 2022). In addition, these authors noted that the decrease in moisture during the ripening process affects the proximal composition of both raw and pasteurized cheeses.

It should be noted that the results of the physicochemical characterization of the cheeses made using isolated strains are preliminary because no manufacturing replicates were carried out.

4 Conclusion

The native LAB strains selected from artisanal Swiss-type cheese from Ancash (Peru) were identified as *L. paracasei* (TQ1, YQ1, EQ1, LQ2, GQ2, KQ3, CQ1), *L. parabuchneri* (BQ2, OQ2, RQ3) and *Lactiplantibacillus* sp. (QQ3). Safety tests demonstrated that the strains did not present health risks because they did not show gelatinase or hemolytic activity. In addition, the antimicrobial activity of *L. paracasei* KQ3, GQ2 and *L. parabuchneri* BQ2 against *S. aureus*, *E. coli* and *L. monocytogenes* was highlighted. Variable susceptibility to antibiotics was also observed. Desirable technological characteristics were evidenced, such as acidifying, proteolytic, lipolytic activity and diacetyl production. As for cheese production with *L. paracasei* CQ1, *L. paracasei* KQ3 and *Lactiplantibacillus* sp. QQ3, an improvement was obtained in terms of flavor, aroma, texture and general appearance after 8 days of ripening. All this demonstrates that the strains isolated from Swiss-type artisanal cheese are safe at the *in vitro* level, have a good technological potential and improve the sensory qualities of the cheeses.

The results open an interesting scenario for the development of new research in cheese formulation using combinations of isolated bacterial strains together with commercial starter cultures, in order to increase sensory acceptability. In addition, it is convenient to study the effect of the ripening period of cheeses inoculated with LAB reported in the present study, on the profiles of volatile compounds and sensory acceptability.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

JV-M: conceptualization, formal analysis, investigation, methodology, writing–original draft, and writing–review and editing. NA-T: formal analysis, investigation, methodology, writing–original draft, and writing–review and editing. EV-P: supervision, investigation, validation and methodology, and writing–review and editing. LR-F: writing–review and editing, research, and visualization. MR-C: writing–review and editing, data curation, and formal analysis. CT-A: methodology, writing–review and editing, and molecular analysis. PO-G: writing–review and editing and molecular analysis. LE-E: methodology, investigation, supervision, writing–original draft, and writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1212229/full#supplementary-material

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