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Bacterial Diversity and Dynamics during Spontaneous Cheese Whey Fermentation at Different Temperatures

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Abstract: The effect of temperature (32–50 °C) on bacterial dynamics and taxonomic structure was evaluated during spontaneous whey fermentation for lactic acid production. Bacterial plate count in fresh whey (5 log CFU/mL) increased in two orders of magnitude after 60 h of fermentation (7 log CFU/mL), followed by one log reduction after 120 h (6 log CFU/mL) at 37 and 42 °C. *Streptococcus* and *Lactobacillus* counts ranged between 5–9 and 5–8 log CFU/mL, respectively. High-throughput sequencing of the 16S rRNA gene (V3-V4 region) used as a taxonomic marker revealed thirteen different bacterial phyla. *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were detected in all fermentation treatments (32–50 °C, 0–120 h), where *Firmicutes* was the predominant phylum. Bacterial diversity included more than 150 bacterial genera with predominant lactic acid bacteria (belonging to *Firmicutes*) such as *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Tetragenococcus*. At the species level, fresh whey presented 61 predominant species (relative abundance > 0.05%); however, only 57.4% of these resisted the fermentation conditions (most of them belonging to lactic acid bacteria genera). *Tetragenococcus halophilus*, *Lactococcus lactis*, and *Enterococcus casseliflavus* were the predominant bacteria found in all treatments. Temperatures between 37–42 °C were more favorable for lactic acid production and could be considered appropriate conditions for fermented whey production and for the standardization of some artisanal cheese-making processes requiring acid whey addition for milk coagulation. The diversity of native beneficial bacteria found in fresh whey offers attractive technological characteristics, and their fermentative capacity would represent a biotechnological option to add value to cheese whey.

Keywords: native bacteria; whey fermentation; whey valorization; bacterial dynamics; lactic acid bacteria

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1. Introduction

Milk, cheese, yogurt, and whey are the most representative dairy products. Whey is a by-product of cheese making which continues to be a discard problem in several countries, including Mexico. Most of the whey discarded belongs to the small and artisanal cheese industry. Several options for its use have been considered; however, these small factories

lack infrastructure, and the technological level of their process is low [1]. Whey quickly deteriorates, especially from raw milk processing, due to its high load of microorganisms and its rich content of nutrients available for their growth. However, raw whey represents a rich source of bacteria with biotechnological potential in food processing (e.g., metabolites production and probiotic properties). The study of indigenous microbiota in fermented foods has led to the development of starters to be used in standardized cheese processes, which also contribute to the hygienic, sensory, nutritional, and functional properties of foods [2].

Natural fermentation represents one of the oldest methods of fermenting foods. Spontaneous fermentation using the native bacteria in raw material has been traditionally used to inoculate a new batch (back-slopping) or used as a starter in producing different fermented foods [3]. Raw materials offer a wide diversity of microorganisms that provide beneficial characteristics in fermented products and can impact food quality and human risk health. Controlling the growth of beneficial indigenous bacteria can produce desired metabolites during fermentation, limiting the production of undesirable compounds that could represent a risk to human health [2].

The use of sour whey produced by spontaneous fermentation represents a common practice in the artisanal production of pasta-filata-type cheeses (e.g., Asadero and Oaxaca). The production of naturally fermented acid whey (NFAW) in artisanal cheese factories is performed at environmental temperature, which can be highly variable depending on the season (20–45 °C); consequently, the time to obtain a required acidity is also variable (1–5 days). Thus, the final lactic acid content and pH of fermented whey are uncertain; therefore, the volume of NFAW used to acidify the milk and cause its coagulation during the cheese-making process is variable [4,5]. The whey obtained from cheese production using raw milk contains a great diversity of bacteria that has been scarcely studied. During NFAW production, lactic acid bacteria metabolize lactose to produce lactic acid, which efficiency depends on fermentation conditions (e.g., temperature and pH) [6]. In addition, native bacteria can cause proteolysis and release bioactive peptides with potential antihypertensive activity or other bioactivities with potential human health benefits [5,7]. The bacteria associated with those changes are unknown since the diversity of native bacteria in fresh whey and its dynamics during NFAW production have not been addressed. The isolation of microbial species with attractive characteristics for technological applications has been performed from several natural niches (e.g., dairy products, meat, vegetables, grains, etc.). Their potential function is due to their fermentative capacity, probiotic properties, production of valuable metabolites, or attractive organoleptic properties to foods [3]. Fermented milk products represent an important category of fermented foods, and whey offers an excellent niche for growing a diverse microbial community. Microbial diversity is sample-specific but can be defined by the dominant microbiota, fermentation conditions, and tolerance of the microbiota to the environmental changes occurring in the fermentation product, among other factors [8]. Therefore, the objective of this study deals with massive sequencing of the bacterial diversity in fresh whey and understanding dynamics occurring during NFAW production under different temperature conditions.

2. Materials and Methods

2.1. Whey Samples and Whey Fermentation

Cheese whey from Queso Fresco production (using non-pasteurized milk) was obtained from an artisanal cheese factory at La Victoria, Hermosillo, Sonora, Mexico. Samples were used within less than 2 h after being drained from cheese curd. Naturally fermented acid whey (NFAW) samples were obtained by incubating 40 mL of fresh whey (in sterile Falcon tubes) at 32, 37, 42, and 50 °C for 0, 60, and 120 h. After fermentation, samples were cooled and kept frozen (−20 °C) until further analyses. The experiment was repeated twice.

2.2. pH Measurement and Lactic Acid Content

The pH and lactic acid content in fresh and fermented cheese whey were determined as described previously [5]. Briefly, pH was measured by direct electrode immersion using

a pH meter 211 (Hanna Instruments, Ciudad de Mexico, Mexico) and lactic acid content by titratable acidity determination. An average of three independent measurements were determined for each sample.

2.3. Mesophilic and Lactic Acid Bacteria Enumeration

The total mesophilic bacterium, streptococcus, and lactobacilli were analyzed following the procedure described by Torres-Llanez et al. [9]. Briefly, whey samples were diluted in saline peptone water, and volumes (0.1 mL) of each dilution were surface plated in the following agar media. Plate count agar was used for total mesophilic bacterium counting after incubation at 37 °C for 24 h. *Streptococcus* were counted on M17 agar (Difco Laboratories, Detroit, MI, USA) after 48 h of incubation at 37 °C. *Lactobacilli* bacteria were counted on MRS agar (Difco Laboratories, Detroit, MI, USA) after 48 h incubation in an enriched CO_2 atmosphere (Anaerobic System; Difco Laboratories, Detroit, MI, USA) at 42 °C.

2.4. DNA Extraction and Amplification

DNA from fresh whey (control) and fermented whey samples was extracted using the commercial kit Power biofilm® (MO BIO Laboratories, Inc., Carlsbad, CA, USA). PCR amplification was performed using the primer sets of the V3-V4 variable region of the bacterial 16S rRNA gene with overhangs 341F: 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG CCTACGGGNGGCWGCAG-3′, and 785R: 5′-GTCTCGTGGGCTCGGAGATGTGTATAAG AGACAG GACTACHVGGGTATCTAATCC-3′.

DNA concentration and quality were measured through microfluidic electrophoresis (2200 Tapestation, Agilent Technologies Inc., Santa Clara, CA, USA).

2.5. Library Construction and Illumina Sequencing

Library construction was performed following the "16S-metagenomic sequencing library preparation guide" of Illumina (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf, accessed on 8 February 2017). Libraries were normalized (2 pM), pooled, and sequenced on the MiSeq sequencing instrument (2 × 300, Illumina, San Diego, CA, USA) following the instructions manual.

2.6. Illumina Data Analysis and Sequence Identification

Sequences obtained from Illumina sequencing were processed for quality control using the MG-RAST platform. Sequences smaller than 200 bp and ambiguous bases > 4, were not considered for the analysis. Taxonomic classification and diversity analysis were performed by comparison against the Ribosomal Database Project using the MG-RAST pipeline. Alpha diversity was estimated by calculating the Shannon index as follows:

$$H = -\sum[(p_i) \times \log(p_i)] \quad (1)$$

where: H—Shannon diversity index; p*i*—the proportion of individuals of *i*-th species in a whole community. p*i* = *n*/N, where: *n*—individuals of a given type/species, and N—total number of individuals in a community.

Principal component analysis displaying the normalized clustered distances of the metagenomes and heatmaps for the most abundant specific species (relative abundance > 0.05) in whey samples was performed in the STAMP software package [10].

3. Results and Discussion

3.1. Viable Bacterial Count in Spontaneously Fermented Whey

The results of viable counts for mesophilic aerobes, lactobacilli, and streptococci during whey fermentation at 37 and 42 °C are shown in Table 1. Total mesophilic aerobes (TPC) in fresh whey were in the range of 5.23–5.29 log UFC/mL and increased around two logs of magnitude after 60 h of spontaneous whey fermentation (SWF) at 37 °C

and 42 °C (7.76 and 7.1 log UFC/mL, respectively). Then, TPC decreased to 6.65 and 5.12 log UFC/mL after 120 h of fermentation at 37 and 42 °C, respectively. *Streptococcus* plate count (M17) increased from 5.08 to 7.37 and 6.63 log UFC/mL values at 37 and 42 °C after 120 h, respectively. De Candia et al. [11] reported similar values for fresh whey (range of 3.6–7.2 log de UFC/mL). On the other side, the maximum counts for *Lactobacillus* (MRS) were observed after 60 h of fermentation for both temperatures, 37 and 42 °C, with 7.99 and 7.27 log UFC/mL, respectively. Fresh whey had an average content of lactose, fat, ash, and protein of 4.8%, 0.50%, 0.4%, and 0.72%, respectively. It was observed that the pH dropped progressively during whey fermentation, decreasing from pH 6.5 (fresh whey) to values in the range of 3.3–3.6 after 60 or 120 h at these temperatures, where lactic acid production was more efficient. In addition, proteolysis (free α-amino content) increased from 36 µg Gly/mL in fresh whey to values in the range of 360–456 µg Gly/mL in fermented whey (37–42 °C, 120 h) [5].

Table 1. Bacterial counts, pH, and lactic acid content in whey fermented at different temperatures and times.

Fermented Whey (Treatment)		Bacterial Counts (log CFU/mL)				
Temperature	**Time (h)**	***Mesophilic* (PCA)**	***Streptococci* (M17)**	***Lactobacilli* (MRS)**	**pH**	**Lactic Acid (g/L)**
37 °C	0	5.29	5.08	4.99	6.49	0.90
	60	7.76	6.87	7.99	3.65	7.42
	120	6.65	7.37	6.21	3.35	12.33
42 °C	0	5.23	5.08	4.74	6.56	0.89
	60	7.01	5.36	7.27	3.35	9.38
	120	5.12	6.63	5.43	3.30	12.83

Samples were analyzed on the same day of sampling.

3.2. Bacterial Phylum Diversity during Spontaneous Whey Fermentation

Bacterial diversity and changes occurring during whey fermentation were assessed by massive sequencing technology. A total of 765,647 reads after quality control were obtained from 24 whey samples and classified into different taxonomic levels (Table 2).

Table 2. Average abundance (%) of sequence assigned to taxa for all whey samples after 0, 60, and 120 h of spontaneous fermentation at 32, 37, 42, and 50 °C.

Fermented Whey (Treatment)	Classified Reads	Phylum	Class	Order	Family	Genus	Species
				%			
32 °C_0 h (Control)	89.95	84.19	81.05	77.93	71.59	54.68	35.36
32 °C_60 h	93.45	86.27	82.88	74.50	70.56	52.09	18.48
37 °C_60 h	92.12	85.76	82.63	77.99	71.23	50.63	35.55
42 °C_60 h	91.82	85.96	84.02	77.36	74.90	38.38	7.73
50 °C_60 h	89.42	85.17	77.84	75.08	66.35	49.68	29.33
32 °C_120 h	92.28	87.66	84.39	81.44	78.41	55.18	40.39
37 °C_120 h	81.87	74.67	69.80	64.39	55.97	39.56	21.87
42 °C_120 h	82.59	76.82	74.26	62.45	60.73	39.22	30.24
50 °C_120 h	84.40	74.93	71.31	69.36	57.38	51.04	40.04

Fresh and fermented whey samples were cooled and kept frozen at −20 °C until analysis.

Thirty phyla were identified, but only *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were predominant in all samples (Figure 1), which could be considered the "core microbiota of whey". The microbiota in fresh whey is expected to be like the milk used and environmental conditions in cheese-making [12,13]. *Firmicutes* was the predominant phylum in fresh whey (89.47%), as well as in most samples of fermented whey. This

bacterial diversity could be related to the optimum growth temperature for most bacteria (30–40 °C) [14]. However, this bacterial distribution was modified during SWF at different temperatures and times (Figure 1). *Firmicutes* predominance decreased, and *Proteobacteria* increased during fermentation at 37 and 42 °C.

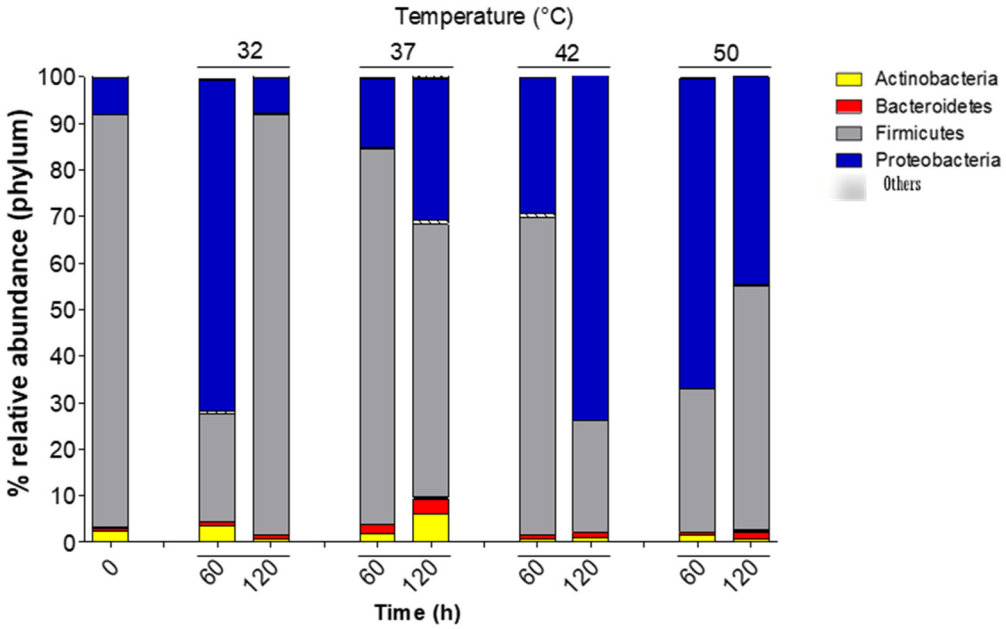

Figure 1. Average abundance (%) of bacterial communities at phylum level found in fermented whey after 60 and 120 h at different temperatures. Others include the phyla *Planctomycetes*, *Spirochaetes*, *Tenericutes*, *Caldiserica*, *Thermotage*, *Chlamydiae*, *Cloroflexi*, *Candidatus Saccharibacteria*, and *Deinococcus-thermus*.

On the contrary, at temperatures out of this range (i.e., 32 and 50 °C), *Firmicutes* first had a reduction and then an increase during fermentation time. In addition to the temperature effect, the fermentative capacity of bacteria and pH tolerance could be associated with bacterial dynamics during whey fermentation since all whey samples were acidic (pH below 3.7) after 60 h. Previous studies observed that the optimum temperature for lactic acid production during spontaneous whey fermentation occurred in the range of 37–42 °C, while at 32 and 50 °C lactic acid production and proteolysis were negatively affected [5]. In this regard, alpha diversity calculated by the Shannon index and registered at 32 and 50 °C (1.58 ± 0.50 and 1.65 ± 0.17) was lower than that registered at 37 and 42 °C (1.91 ± 0.53 and 2.04 ± 0.35), suggesting the lowest and highest temperatures evaluated in this trial may not be adequate for many material members of the whey microbiota.

The predominance of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* has also been reported in Poro Cheese, adding that Firmicutes was predominant after cheese fermentation [15]. Similarly, naturally fermented sour whey (3–5 days) showed a similar distribution of bacterial communities where Firmicutes and Proteobacteria were the predominant phyla in three of four regions of China [8]. On the other side, Lusk et al. [16] reported that *Proteobacteria* and *Firmicutes* were predominant in different brands of Latin-style cheeses (e.g., queso fresco).

In this study, principal component analysis at the phylum level was able to separate the unfermented fresh whey sample (control) from spontaneously fermented whey samples produced at different fermentation conditions (temperature and time) (Figure 2). All analyzed unfermented fresh whey samples (n = 8) formed a cluster indicating a high similarity. However, after fermentation (60 and 120 h), whey samples were located out of this cluster. Samples fermented at 37 and 42 °C showed higher similitude among them but different from 32 and 50 °C, which were also different among them. These differences in bacterial diversity amongst treatments (fermentation temperature) would

be related to the thermal conditions established and the subsequent conditions caused by the metabolic activity of bacteria capable of optimally growing under the different temperatures, producing lactic acid at different rates, causing proteolysis, and resisting the acidified conditions [5].

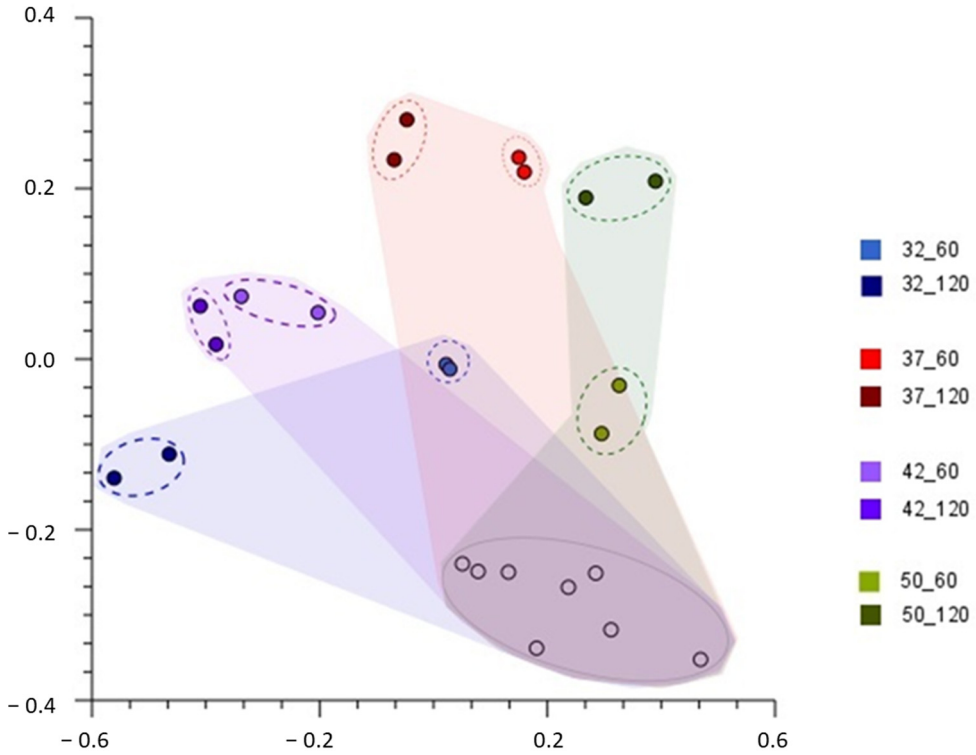

Figure 2. Principal component analysis plot of bacterial communities at the phylum level. Whey samples were fermented by their native microbiota at 32, 37, 42 and 50 °C for 0, 60, and 120 h. Non-colored circles correspond to unfermented fresh whey samples.

The proportion of other phyla (i.e., *Planctomycetes*, *Spirochaetes*, *Tenericutes*, *Caldiserica*, *Thermotage*, *Chlamydiae*, *Cloroflexi*, *Candidatus Saccharibacteria*, and *Deinococcus-thermus*) was slightly affected by the spontaneous whey fermentation process since their abundance as a group was always lower than 3%. These other phyla could be considered non-fermentative bacteria (NFB). These NFB are not related to lactic acid production during whey fermentation; however, their presence is relevant in the production of beneficial compounds that could contribute to the stability of the microbiota or with bacterial nutrition, stability, cellular signaling, or other important functions for the microbial community balance [17]. The presence of NFB has been reported in other dairy products such as Suero Costeño and a surface-ripened semihard Danish Danbo cheese [18,19].

3.3. Bacterial Diversity during Whey Fermentation at Genus Level

Around 150 genera were identified in all whey samples, but only 16 were found representative according to their predominance, where the lactic acid bacterium (LAB) *Streptococcus*, *Lactobacillus*, *Lactococcus*, and *Tetragenococcus* were the most predominant (Table 3). These LAB genera could be more related to the lactic acid fermentation occurring during spontaneous whey fermentation; however, the presence of non-fermentative bacteria from other genera such *Acinetobacter*, *Bacillus*, *Macrococcus*, and *Nocardioides* could also be important during the fermentative process [17,18].

Table 3. Relative abundance (%) of the bacterial community in whey during spontaneous lactic acid fermentation at the genus level.

Genus of Lactic Acid Bacterium		Genus of Non-Fermentative Bacterium			
		Staphylococcus	9.5	*Salmonella*	3.7
Streptococcus	17.2	*Alteromonas*	8.0	*Macrococcus*	3.5
Lactococcus	11.2	*Geobacillus*	7.7	*Pantoea*	3.5
Lactobacillus	8.0	*Acinetobacter*	4.5	*Erwinia*	3.2
Tetragenococcus	5.5	*Arcanobacterium*	4.2	*Bacillus*	3.0
		Ketogulonicigenium	3.7	*Nocardioides*	3.0

Values for relative abundance (%) correspond to the average considering all treatments.

Lactococcus has been reported as the most abundant genera in raw milk and soft cheese [20]. However, *Lactobacillus* and *Lactococcus* were reported as the dominant genera in sour whey, accounting for 63.1% and 19.4% of the total bacteria count, respectively [8].

3.4. Species Bacterial Diversity in Fermented Whey

The temperature must significantly affect bacterial diversity during spontaneous whey fermentation and be determinant for the predominance of some bacteria during lactic acid production. Principal component analysis at specie level after 120 h of fermentation (Figure 3) indicated a clear differentiation among the treatments (temperature). It can be observed that whey fermentation at 50 °C had a significant effect on the taxonomic profile of the microbiota in comparison with other fermentation temperatures. This effect could be related to the thermal tolerance of some prokaryotic species causing their thermal death or reducing their replication rate. The negative effect of high temperature on predominant bacteria could favor the growth of thermotolerant bacteria [21]. Surprisingly, Figure 3 shows a significant similarity of the microbiota, at the species level, between 32 °C and 42 °C, than at 32 °C and 37 °C, which could indicate that the optimum temperature for predominant bacteria is close to 37 °C (see the first five bacteria in Table 4).

Bacterial dynamics for predominant bacteria at the species level can be observed in the heat map in Table 4. A total of 61 species were identified in fresh whey (time zero); however, 42.63% of their prevalence was lost during the spontaneous whey fermentation since no detection after fermentation treatment occurred.

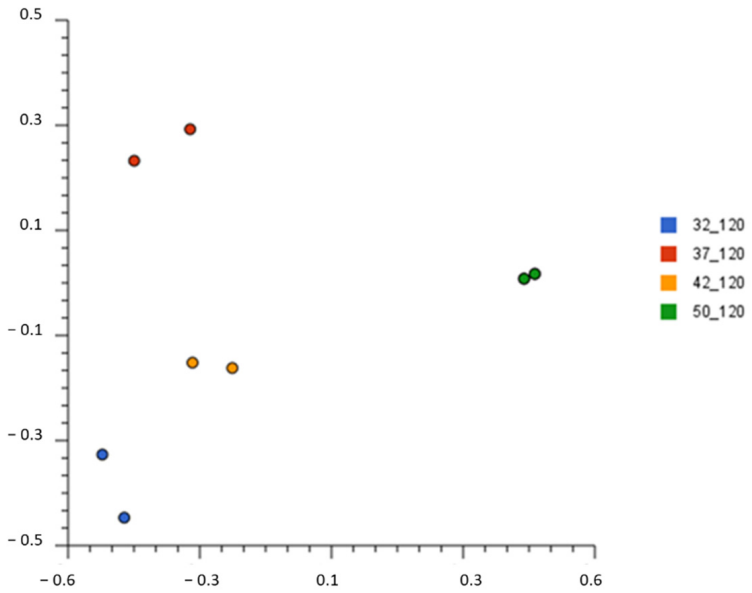

Figure 3. Principal component analysis of whey microbiota (at specie level) after 120 h of fermentation at 32, 37, 42, and 50 °C. The distance among points is inversely proportional to microbiome similarity.

Table 4. Heatmap displaying the abundance of predominant bacterial species during whey fermentation for 60 and 120 h at different temperatures.

		Spontaneous Fermented Whey							
		60 h				120 h			
		Temperature (°C)							
Bacteria	**Fresh Whey**	**32**	**37**	**42**	**50**	**32**	**37**	**42**	**50**
Alteromonas macleodii									
Streptococcus agalactiae									
Tetragenococcus halophilus									
Arcanobacterium haemolyticum									
Geobacillus thermoleovorans									
Lactococcus lactis									
Staphylococcus aureus									
Rhodopirellula baltica									
Sulfurimonas autotrophica									
Enterococcus casseliflavus									
Pantoea vagans									
Brachybacterium faecium									
Streptococcus salivarius									
Streptococcus thermophilus									
Cytophaga hutchinsonii									
Macrococcus caseolyticus									
Ketogulonicigenium vulgare									
Bacillus subtilis									
Lactobacillus sakei									
Deinococcus gobiensis									
Erwinia pyrifoliae									
Bacillus cereus									
Jannaschia sp. *CCS1*									
Lactobacillus salivarius									
Lactobacillus acidophilus									
Lactobacillus reuteri									
Marivirga tractuosa									
Plautia stali symbiont									
Microbacterium testaceum									
Sphingobacterium sp.*21*									
Salmonella enterica									
Acinetobacter baumannii									
Streptococcus mutans									
Lactobacillus helveticus									
Riemerella anatipestifer									
Lactococcus garvieae									
Croceibacter atlanticus									
Ilumatobacter coccineus									
Dinoroseobacter shibae									
Escherichia coli									
Salmonella bongori									
Erysipelothrix rhusiopathiae									
Ruegeria pomeroyi									
Secondary endosymbiont of Heteropsylla cubana									
Moraxella catarrhalis									
Kucuria rhizophila									
Leuconostoc mesenteroides									
Dickeya dadantii									
Candidatus Moranella endobia									

Relative abundance (%) <0.01 | 0.01–0.05 | 0.05–0.1 | 0.1–0.5 | 0.5–1 | 1–5 | >5 .

Bacteria identified in cheese whey appearing at least in one treatment with a relative abundance higher than 0.05%.

Among them, ten bacteria from *Firmicutes* (*B. amyloliquefaciens*, *B. thuringiensis*, *B. brevis*, *C. botulinum*, *C. perfringens*, *E.* sp. *AT1b*, L. *fermentum*, *P. difficile*, *S. carnosus*, and *S. lugdunensis*), six *Proteobacteria* (*A. pasteurianus*, *C. zinderia insecticola*, *K. oxytoca*, *P.* sp. *PRWF-1*, *W. glossinidia,* and *V. parahaemolyticus*), two *Actinobacteria* (*A. subflavus* and *T. bispora*) and five *Deinococcus-Thermus* (*D. radiodurans*, *O. profundus*, *M. fermentans*, *W. chondrophila*, and *P. brasiliensis*) were lost during the fermentation process (Data not shown).

On the contrary, nine bacteria (i.e., *S. autotrophica*, *L. salivarius*, *S. enterica*, *D. shibae*, *Secundary endosymbiont* of *H. cubana*, *M. catarrhalis*, *L. mesenteroides*, *D. dadantii*, and *C. mortadella endobia*) increased in abundance (>0.05%) during fermentation in some temperature conditions that favored their growth (Table 4). The changes associated with the bacterial dynamic during spontaneous whey fermentation could be associated mainly with the temperature, which is the optimum temperature determinant for the species' growth and survival. However, other changes in the media associated with pH drop and lactic acid content also can affect the prevalence of some specific bacteria. Optimum lactic acid production was in the range of 37–42 °C [5], which could be associated with the LAB *S. thermophilus* and *L. lactis*, since their growth condition was satisfactory.

On the contrary, the bacterial growth at 32 °C was slower, while at 50 °C the bacterial growth was more negatively affected, hence the bacterial diversity (Table 4). In the same way, *B. faecium*, *L. garvieae*, *L. mesenteroides*, *L. acidophilus*, *S. entérica*, and *S. thermophilus*, were drastically affected at 50 °C since these species were not detected after 60 h of fermentation. This negative effect on some bacteria could also be related to their low tolerance to acid pH, such as the enteric genera *Salmonella* and *Escherichia*. In contrast, BALs have an optimum growth pH in the range of 4.5–7.0 and can survive at the acidic pH of 3.3–3.6 found in sour whey [14,22].

Next-generation sequencing was able to study the dynamic of LAB bacteria during spontaneous whey fermentation for lactic acid production. The presence of unexpected bacteria from the marine environment, such as *A. macleodii* and *R. baltica* is not surprising since species from this niche, such as *Pseudoalteromones*, have been reported in smear-ripened cheese and the core [20]. In addition, the presence of some bacteria with probiotic potentials, such as *L. reuteri* and *L. helveticus*, as well as others with technological properties such as *G. thermoleovorans* (lipase-producer) [23] support the hypothesis that fermented whey produced by its native bacteria represent an attractive niche for the isolation and production of bacterial species for further applications in biotechnological process.

4. Conclusions

Firmicutes, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* could be considered the whey "core microbiota" regardless of the fermentation at different temperatures. However, the incubation temperature affected the overall bacterial diversity during spontaneous whey fermentation. LABs from the genus *Enterococcus*, *Lactobacillus*, *Lactococcus*, and *Streptococcus* were predominant in fresh and fermented whey. The presence of other non-lactic bacteria, such as *B. subtilis*, *G. thermoleovorans*, *S. aureus*, *S. salivarius*, *A. macleodii,* and *K. vulgare* (from marine origin), was not associated with lactic acid production; however, their presence could contribute to the growth of other bacteria. Lactic acid production during whey fermentation affected the presence of pathogens genera such as *Clostridium* and *Salmonella* (0.05–0% predominance), which could be associated with their low tolerance to acidic conditions in fermented whey. LAB predominated in spontaneously fermented whey, produced under optimum conditions for lactic acid production (37–42 °C); therefore, its use for milk acidification to produce some pasta-filata-type cheeses (e.g., Oaxaca and Asadero), represent an attractive option to use the whey in artisanal cheese factories. In addition, fresh and fermented whey represent a niche rich in bacteria with probiotic and technological properties.

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