Study of the bacterial profile of raw milk butter, made during a challenge test with *Listeria monocytogenes*, depending on cream maturation temperature

Soundous El-Hajjaji[1], Amaury Gérard[1], Juliette De Laubier[1], Aurélie Lainé[1], Viviane Patz[1], Marianne Sindic[1]

[1] Laboratory of Quality and Safety of Agro-food Products, Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés, 2, 5030 Gembloux, Belgium.

Corresponding author:

El-Hajjaji Soundous (email: soundous.el-hajjaji@student.uliege.be)

University of Liège - Gembloux Agro-Bio Tech

Passage des Déportés, 2

B-5030 Gembloux.

Abstract

Bacteria can play different roles and impart various flavors and characteristics to food. Few studies have described bacterial microbiota of butter. In this study, next-generation sequencing was used to determine bacterial content of raw milk butter, processed during a challenge test, depending on cream maturation temperature and on the presence or not of *L. monocytogenes*. Two batches were produced. pH and microbiological analyses were conducted during cream maturation and butter storage. DNA was also isolated from all samples for 16S rRNA amplicon sequencing analysis. For butter made from cream matured at 14 °C, a growth potential of *L. monocytogenes* of - 1.72 log cfu/g was obtained. This value corresponds to the difference between the median of counts at the end of storage and the median of counts at the beginning of storage. This butter (pH value of 4.75 ± 0.04) was characterized by a dominance of *Lactococcus*. The abundance of *Lactococcus* was significantly higher in inoculated samples than in control samples (p value <0.05). Butter made from cream matured at 4 °C (pH value of 6.81 ± 0.01) presented a growth potential of 1.81 log cfu/g. It was characterized by the abundance of psychrotrophic bacteria mainly *Pseudomonas.* This study demonstrated that cream maturation temperature impacts butter microbiota, affecting thus product's characteristics and its ability to support or not the growth of pathogens like *L. monocytogenes*.

Key words: 16S rRNA sequencing, metagenetics, growth potential

1. Introduction

Listeria monocytogenes is the causative agent of listeriosis, a severe foodborne disease with high mortality rate. In 2018, 229 deaths were reported in Europe due to listeriosis, representing a case fatality rate of 15.6% (European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC), 2019). Most cases of listeriosis arise from the ingestion of contaminated food, especially ready-to-eat (RTE) (Jofré et al., 2016; Pérez-Rodríguez et al., 2017).

As a RTE food, butter is also prone to contamination by *L. monocytogenes*. However, its ability to support survival or growth of the pathogen depends on its formulation and characteristics (Holliday et al., 2003; Michelon et al., 2016; Voysey et al., 2009). Many intrinsic and extrinsic factors such as temperature, pH and water activity (a_w), were shown to affect the growth of *L. monocytogenes* in food (Fernandez et al., 1997; Hayman et al., 2008; Nyhan et al., 2018; Schvartzman et al., 2011). RTE foods with pH $\leq$ 4.4 or $a_w \leq 0.92$ or pH $\leq$ 5.0 and $a_w \leq 0.94$ do not support the growth of *L. monocytogenes* (Commission Regulation (EC), 2005). In Wallonia (Belgium), no growth of *L. monocytogenes* was observed during storage of naturally contaminated samples of raw milk butter, even though they presented pH and a_w values theoretically allowing the growth of the pathogen (El-Hajjaji et al., 2020).

The presence of antimicrobials or competitive microbiota can also inhibits the growth of *L. monocytogenes* (Al-Zeyara et al., 2011; Brandt et al., 2010; Goerges et al., 2006; Murdock et al., 2007). Lactic acid bacteria (LAB), for example, have shown an inhibitory effect on *L. monocytogenes* in various food matrices (Amézquita and Brashears, 2002; Arqués et al., 2005; Koo et al., 2012; Teixeira de Carvalho et al., 2006). To study food microbiota, traditional methods based on cultivation, isolation and identification of bacteria based on their morphological characteristics were often used. Nowadays, newer and automated methods are adopted, including sequencing of the 16S rRNA gene (Phumudzo et al., 2013). Over the past

decade, next-generation sequencing technologies evolved rapidly and led to an improved representation of samples biodiversity (Shokralla et al., 2012).

To our knowledge, published studies of food microbial ecology have focused on plant-, meat- and fish-derived fermented foods, milk, fermented milk and cheese. Studies of bacterial communities of butter have rarely been conducted. The objective of this study was to use next-generation sequencing to analyze bacterial content of raw milk butter, processed during a challenge test, depending on cream maturation temperature and on the presence or not of artificially inoculated *L. monocytogenes*.

2. Materials and methods

2.1. *Listeria monocytogenes* cultures

To consider the growth variability between strains, a cocktail of two strains (ATCC 19114 and 12MOB105LM of a culture collection, provided by Quality Partner sa (Herstal, Belgium)) was used in this study. The second strain was isolated from a dairy product. Cryobeads containing respective strains were incubated at 37 °C for 18 h in 9 ml brain heart infusion (BHI). A subculture was prepared by diluting 1 ml of this culture into 9 ml of BHI and incubated at 7 °C for 7 days. A cocktail was prepared by mixing the same volume from each culture. Dilutions of the mixed cultures were then made until obtaining a concentration of 10^5 cfu/ml.

2.2. Butter manufacture

Two batches of raw milk butter were manufactured in a pilot unit (Food Science Department, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium). The batches were produced at the same day and using the same batch of cream obtained from a dairy farm directly after skimming. For each batch of butter, 20 l of cream were used. Half of the cream was inoculated with 5 ml of the cocktail of strains to obtain a contamination level of 50 cfu/ml. Remaining cream was used to manufacture control samples. Creams were then

incubated for 3 days of maturation, either at 4 °C (first batch, B1) or at 14 °C (second batch, B2). These two temperatures of maturation were selected to represent the two most common and opposite practices (maturation in fridge (4 °C) or workshop (14 °C)) followed by raw milk butter producers in Wallonia (El-Hajjaji et al., 2019). The matured creams were churned until phases' separation. After buttermilk removal, grains of butter were washed three times with cold water (12 to 14 °C) and finally kneaded and packed into blocks of 250 g. Butter was stored at 9 °C for 30 days. No starter cultures were added so as not to affect the initial microbiota. It is also the most adopted practice in Wallonia (El-Hajjaji et al., 2019). No salt was added neither.

2.3. Microbiological and physico-chemical analyses

For all analyses, three different samples of cream and/or butter per batch were submitted each time. All samples (inoculated and non-inoculated) were analyzed for total mesophilic microbiota, LAB and pH according to ISO 4833, ISO 15214 and ISO 2917 methods, respectively. Analyses were conducted at D'0 (before maturation), D'1 (after 1 day of maturation), D'2 (after 2 days of maturation) and D'3 (after 3 days of maturation) for cream samples and at D0 (before storage), D7 (after 7 days of storage), D14 (after 14 days of storage) and D30 (after 30 days of storage) for butter samples. For the latter samples, a_w was also determined at the beginning (D0) and the end of the storage period (D30), using the ISO 21807 method.

L. monocytogenes was enumerated in inoculated samples at D'0, D'1, D'2 and D'3 for cream, and D0, D7, D14 and D30 for butter. The enumeration was conducted according to RAPID' L.mono (Bio-Rad, Hercules, CA, USA) method with a detection limit of 10 cfu/g. For control samples, only the detection of the bacteria was performed at the beginning of cream maturation and at the beginning of butter storage.

2.4. DNA extraction and sequencing

DNA extraction and sequencing were carried out on three different samples of cream and/or butter per batch each time. DNA was isolated from each sample using the FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's recommendations. DNA was eluted into DNase-free water and its concentration and quality were evaluated using a NanoDrop ND-1000 spectrophotometer (ThermoFisher, Wilmongton, USA).

DNA samples were stored at -20 °C until use in 16S rRNA gene amplicon sequencing analysis.

Library preparation and sequencing analysis were carried out by DNA Vision S.A. (company, Gosselies, Belgium) using Illumina technology. Library preparation was done by amplifying the V1-V3 region of the 16S rRNA gene. The forward and reverse primer sequences used in this study, including the Illumina adapters, were 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCCTGGCTCAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG-3', respectively.

2.5. Bioinformatics analysis

The analysis of the sequencing data was conducted using Mothur software package for trimming, length and quality filtering, and the removing of chimeras (Schloss et al., 2009). The sequences that passed the quality check were aligned to the SILVA alignment database at genus level (Quast et al., 2012). The final reads were then clustered into operational taxonomic units (OTUs) at a 0.03 distance unit cutoff.

2.6. Statistical analysis

Calculation of the growth potential: The growth potential (δ) is the difference between the median of the log cfu/g counts at the end of the storage and the median at the beginning (EURL *Lm* method). If δ is higher than 0.5 log cfu/g, it is assumed that the food is able to support the growth of *L. monocytogenes*, and *vice versa* if the δ is lower than 0.5 log cfu/g

(Beaufort et al., 2014). The growth potential was also calculated using the FASFC method (2019) reported by Gérard et al. (2020) as the difference between the highest value at the end of storage and the lowest at D0 (FASFC method).

Bacterial diversity: To evaluate bacterial richness and diversity of the samples, data sets were subsampled using Mothur to obtain the same number of reads per sample. Richness was assessed using number of OTUs and Chao1 estimator, while diversity was assessed using the Shannon diversity index and Inverse Simpson index.

Bacterial population dissimilarity: Difference of profiles was examined using non-metric multidimensional scaling (NMDS) based on Yue & Clayton theta index (Yue and Clayton, 2005). Statistical differences in the bacterial populations between samples were highlighted using analysis of molecular variance (AMOVA). Differences were considered significant when p-values were lower than 0.05. The function "metastats" of Mothur software was then used to determine which OTUs were differentially represented between the samples.

3. Results

3.1. Characterization of creams and butters

The physico-chemical and microbial characteristics of creams and butters during maturation and storage are summarized in Tables 1a and 1b, respectively.

At the beginning, the pH values of the two batches of cream were 6.77 ± 0.01 and 6.75 ± 0.01, respectively. During maturation, the pH of B2 cream (maturation at 14 °C) decreased significantly compared to B1 (maturation at 4 °C). The values obtained at the end of maturation were 4.58 ± 0.01 and 6.76 ± 0.01, respectively. After churning, washing and kneading, pH values undergo a slight increase reaching 4.75 ± 0.04 and 6.81 ± 0.01 for B2 and B1 butters, respectively. During storage, both B1 and B2 butter samples showed a decrease in pH. At the end of the storage period, average pH was 4.52 ± 0.02 and 5.39 ± 0.03 for B2 and B1, respectively. Regarding a_w, the two batches presented a value of 0.98 ± 0.00.

Regarding the behavior of total microbial counts and LAB during cream maturation, results showed a gradual increase in the samples of B1, to reach a mean value of 7.25 ± 0.04 and 5.13 ± 0.11 log cfu/g at the end of maturation, respectively. However, levels of total microbial counts and LAB in B2 samples increased suddenly after one day of maturation to reach 8.30 ± 0.00 and 8.03 ± 0.47 log cfu/g, respectively.

In control butters, levels of total microbial counts and LAB increased by 1.26 (from 5.46 to 6.72 log cfu/g) and 2.31 log units (from 4.43 to 6.75 log cfu/g) in B1 samples during storage, respectively, while it decreased by 1.1 (from 7.31 to 6.21 log cfu/g) and 0.76 log units (from 7.44 to 6.68 log cfu/g) in B2 samples, respectively.

3.2. Growth potential of *L. monocytogenes*

As shown in Table 1a, the level of *L. monocytogenes* increased by 1.56 log cfu/g after maturation for cream stored at 4 °C (B1) and by 3.43 log cfu/g for cream stored at 14 °C (B2). The levels obtained for the two batches were 3.75 ± 0.06 and 5.18 ± 0.04 log cfu/g, respectively. After production, a decrease in contamination levels was observed. The levels of *L. monocytogenes* in butter samples at D0 were respectively 1.49 ± 0.43 log cfu/g and 3.34 ± 0.64 log cfu/g for B1 and B2 (Figure 1).

The representation of the behavior of *L. monocytogenes* in butters during storage is presented in Figure 1. After 30 days of storage, growth potentials of 1.81 and 2.60 log cfu/g were obtained for B1 butter using EURL *Lm* and FASFC methods, respectively. This product therefore allowed the growth of *L. monocytogenes* unlike B2 butter. The second batch presented growth potentials of - 1.72 (EURL *Lm* method) and -1.47 log cfu/g (FASFC method). *L. monocytogenes* was not detected in control samples.

3.3. Bacterial diversity in cream and butter

The number of OTUs, the bacterial diversity and richness estimators according to type of samples are presented in Tables 2a and 2b. The highest number of OTUs in all cream samples

was encountered at D'0. However, B2 cream samples (maturation at 14 °C) showed a decrease in number of OTUs throughout maturation, while the number remained relatively high in B1 cream samples (maturation at 4 °C). B2 cream samples also showed a low diversity at the end of maturation compared to B1 cream samples.

The difference between the two batches continued to be observed in butter samples. The number of OTUs and diversity indices were higher in B1 than in B2 butter samples.

3.4. Bacterial composition of cream and butter

As no co-sequencing of mock communities was conducted, the error rate due to the biases introduced in sequencing was not assessed. The presented results are thus an estimation of the community composition of the samples.

Three major bacterial phyla (Proteobacteria, Firmicutes and Bacteroidetes), representing more than 90% of relative abundance, were identified in all samples. In B1 cream samples, Proteobacteria were dominant throughout maturation with a continuous increase of their relative abundance to reach 85% at D'3. The same result was observed in B2 cream samples for the first two days. However, at D'2 the relative abundance of Firmicutes increased significantly to reach 80% at D'3. There were no significant differences in bacterial relative abundance between blank and inoculated samples. The dominance of Proteobacteria and Firmicutes continued to be observed in B1 and B2 butter samples during storage, respectively.

At the genus level (Figure 2), 138 bacterial genera were detected in cream samples before maturation (D'0) of which 22 had an average relative abundance ≥ 1%, representing 72% of the total reads. *Undibacterium* (11%), *Ralstonia* (8%), *Acinetobacter* (6%), *Lactococcus* (4%), *Burkholderia* (3%) and *Aeromonas* (1%) were among the most abundant. After the first day of maturation, the bacterial profiles for B1 and B2 cream samples were different. For B1 cream samples, percentages of reads of *Acinetobacter*, *Pseudomonas* and *Aeromonas* increased during maturation to reach at the end 30%, 12% and 9% of relative abundance,

207 respectively. In terms of relative abundance, these major genera were followed by
208 *Lactococcus* (5%), *Undibacterium* (3%) and *Ralstonia* (2%). As for B2 cream samples, the
209 number of genera detected at the end of maturation was half that of B1 cream samples (32 and
210 66, respectively) with the dominance of *Lactococcus* (74%) followed by *Acinetobacter* (8%)
211 and *Aeromonas* (4%).
212 In butter samples (Figure 3), there were more genera detected in B1 than in B2 samples. After
213 production, 69 bacterial genera were detected in B1 butter samples, of which 15 were more
214 abundant (with average relative abundance ≥ 1%) namely *Acinetobacter* (15%), *Pseudomonas*
215 (12%), *Lactococcus* (12%), *Undibacterium* (9%) and *Ralstonia* (7%). As for B2 butter
216 samples, 36 genera were identified of which 9 presented an average relative abundance ≥ 1%.
217 Representing 73% of the total reads, *Lactococcus* was the most abundant one. During storage,
218 psychrotrophic bacteria, mainly *Pseudomonas* increased to be the most dominant in B1 butter
219 samples, while *Lactococcus* continued to be dominant in B2 butter samples.
220 There were no significant differences in bacterial profile between blank and inoculated
221 samples (AMOVA, p value 0.6). However, the abundance of *Lactococcus* was significantly
222 higher in B2 inoculated samples than in blank samples (p value <0.05).
223 3.5. Comparison of the bacterial community of samples
224 As shown in Figure 4, dissimilarity test based on Yue & Clayton theta distance revealed that
225 the community difference between B1 and B2 butter samples was significant (AMOVA, p
226 value < 0.001). Analyzed results revealed that this significant difference could be owed to the
227 abundance of *Lactococcus* in B2 samples. In contrast, *Acinetobacter* and *Pseudomonas* were
228 more abundant in B1 samples. Dissimilarity test also showed a difference within B1 butter
229 samples linked to the day of analysis except between D7 and D14 (p value 0.247). This
230 difference could be due to the increase in abundance of *Pseudomonas* during storage.

231 4. Discussion

The objective of this work was to study the bacterial flora of raw milk cream and butter during production, depending on cream maturation temperature and on the presence or not of *L. monocytogenes*. Metagenetics results showed that cream and butter microbiota varied significantly between the two batches made from creams matured at 4 °C (B1) and 14 °C (B2), respectively. The first batch (B1) was mainly characterized by the presence of *Pseudomonas* and *Acinetobacter*, with an increase of their relative abundances during cream maturation at 4 °C and butter storage at 9 °C. As psychrotrophic microorganisms, these bacteria grow well even at 4 °C (Hébraud and Potier, 1999; Oliveira et al., 2015; Perin, 2012). In a study conducted by Raats et al. (2011), the abundance of these two genera in milk samples from dairy plant tank, where it was stored at 4 °C for 54 h at time of sampling, was higher than in those from farm bulk tank (stored at 4 °C for 22 h). The dominance of these Gram negative bacteria in dairy tank milk was also observed by Fricker et al. (2011). Contrary to *Pseudomonas* and *Acinetobacter*, the relative abundance of *Lactococcus* in B1 samples decreased during storage. Refrigeration had an effect on the representation of *Lactococcus* (Lafarge et al., 2004). *Lactococcus* is a mesophilic bacterium with a minimum growth temperature of 5 to 10 °C, hence its representation was low in B1 samples (Anonymous, 2003).

Unlike B1 samples, *Lactococcus* was highly abundant in B2 samples (70% of the total reads). *Lactococcus* belongs to LAB, a group of Gram positive bacteria involved in food fermentation by converting glucose to lactic acid (Stiles and Holzapfel, 1997). LAB is a dominant population in raw milk (Montel et al., 2014; Quigley et al., 2013). Besides *Lactococcus*, the most common LAB genera found in milk are *Lactobacillus, Streptococcus*, *Leuconostoc* and *Enterococcus*. These bacteria are also observed in dairy products (Cogan et al., 1997; Delcenserie et al., 2014; Jayashree et al., 2013; Liu et al., 2015; Yu et al., 2011). However, their representation differs depending on products, production environments and

processes. In our study, low relative abundance was detected in cream and butter samples for LAB other than *Lactococcus*. A similar result was reported by Yu et al. (2018) who found that 77.73% of the total reads corresponded to *Lactococcus*, which was thus the most dominant genera in butter samples. In another study conducted by Guessas et al. (2012) on traditional butter (Dhan) made from unpasteurized fermented milk, *Lactobacillus* (46.05%) was the most dominant genera, followed by *Enterococcus* (26.32%), *Lactococcus* (17.11%) and *Leuconostoc* (10.53%). The dominance of species of *Lactobacillus* in butter samples, made from pasteurized milk cream, was also described by Syromyatnikov et al. (2020).

Besides of the dominant genera, other bacteria with relative abundance ≥ 1% were detected. *Undibacterium*, which was never observed in butter, was identified in the two batches. *Undibacterium* are Gram negative bacteria that are often isolated from water (Kämpfer et al., 2007; Kim et al., 2014), which can explain their occurrence in butter. Species of *Undibacterium* were also isolated from soil and feces of cattle (Kim and Wells, 2016; Kim et al., 2014). In fact, water, soil and feces, among other environments, are rich sources of microorganisms and a direct or indirect transfer of cells to milk and dairy products is frequent (Montel et al., 2014; Perin et al., 2019; Quigley et al., 2013). A species of *Undibacterium* was detected in pasteurized milk (Garofalo et al., 2017).

Ralstonia is another uncommon genus which was detected in this study. Like *Undibacterium*, this genus presented high relative abundances in B1 than in B2 butter samples. *Ralstonia* are plant-associated bacteria that are known as important phytopathogens (Gnanamanickam, 2007). However their presence in raw milk and cheese has already been observed (Delbes et al., 2007; Fricker et al., 2011; Kuehn et al., 2013; Salazar et al., 2018). Species of *Ralstonia* were also detected in buttermilk (Jayashree et al., 2013).

Burkholderia, other bacteria that occur in plants, were found in B1 butter. They were formerly classified in the genus *Pseudomonas* (Gnanamanickam, 2007). Species of the genus

Burkholderia occupy diverse ecological niches including the rhizosphere of plants, water and soil (Coenye and Vandamme, 2003), and can thus be introduced into raw milk (Moore et al., 2001; Saad and Amin, 2012). The presence of *Ralstonia* and *Burkholderia* among other bacteria found in soil and water could also be due to the contamination of DNA during extraction by the kit reagents (Salter et al., 2014). PCR reagents are another source of DNA contamination (Corless et al., 2000; Grahn et al., 2003; Salter et al., 2014). PCR can also lead to other errors which may affect sequencing results (Potapov and Ong, 2017). In this study, the error rate due to PCR amplification and sequencing was not assessed.

Raw milk microbiota may also contain *Aeromonas* (Benner, 2014; Quigley et al., 2013), which was detected in the studied butters. This genus was also observed in other dairy products including fermented milk, buttermilk, yoghurt and cheese (ElBalat et al., 2014; Jayashree et al., 2013; Liu et al., 2015).

Microorganisms can play either a positive or a negative role in food. LAB are widely recognized as food preservatives. Their production of lactic acid results in pH reduction (Caplice, 1999; Widyastuti et al., 2014). In the current study, pH of the second batch of butter (4.75 ± 0.04) was significantly lower than pH of the first batch (6.81 ± 0.01). The former had LAB counts higher than the latter (Table 1b). pH is an important factor for the growth of microorganisms. The growth of *L. monocytogenes* is possible at pH values between 4.4 and 9.6 (Magalhães et al., 2014). Based on this, the growth of *L. monocytogenes* was supposed possible in the two batches of butter studied in this paper. However, the results showed that the bacterium did not grow in butter samples from the second batch ($\delta = -1.72$ log cfu/g). This finding was in accordance with a previous study where no growth of *L. monocytogenes* was observed in naturally contaminated raw milk butter samples, presenting an average pH value of 5.12 ± 0.47 at the beginning of storage (El-Hajjaji et al., 2020). The second batch in the present study was characterized by a dominance of *Lactococcus*, a genus of LAB. The

abundance of *Lactococcus* was even higher in samples containing *L. monocytogenes* compared to control samples. Besides reducing pH, lactic acid has an inhibitory effect on the growth of microbial pathogens, including *L. monocytogenes* (Anang et al., 2007; Ariyapitipun et al., 2000; Lin et al., 2002; Wang et al., 2015). LAB also produce bacteriocins, substances possessing antimicrobial activities (Dortu and Thonart, 2009; Soomro et al., 2002).

5. Conclusion

This study was conducted to analyze bacterial flora of raw milk butter depending on cream maturation temperature. The two batches studied showed a different bacterial profile with a much more diversity in butter made from refrigerated matured cream. This butter was characterized by an abundance of psychrotrophic bacteria mainly *Pseudomonas* while butter made from acidic cream was dominated by *Lactococcus* bacteria. Besides, the growth of *L. monocytogenes* was not observed in this batch. It was also observed that the abundance of *Lactococcus* was even higher in the second batch samples containing *L. monocytogenes* compared to control samples. The temperature of cream maturation has a strong influence on raw milk butter subdominant microbiota, which can affect the growth of pathogenic bacteria like *L. monocytogenes*.

As this study was conducted on one batch as a first experiment to draw hypotheses, it would be interesting to work on other batches to confirm the results regarding the growth of *L. monocytogenes* following the two conditions of cream maturation.

Metagenetic analysis was a first approach to explain the different behavior of *L. monocytogenes* in the two batches. Further studies should be performed in order to assess the real difference in community composition between the samples. It would be interesting to conduct a co-sequencing of mock communities to assess the error rate due to the biases introduced in PCR amplification and sequencing.

Acknowledgments

The authors are thankful to Quality Partner SA and DNA Vision SA for carrying out the analyses and sequencing. They are also thankful to professor Georges Daube of the Faculty of Veterinary Medicine (University of Liège, Liège, Belgium), for giving the permission to use the pilot unit of the Food Science Department. They also thank professor Sébastien Massart of the Faculty of Gembloux Agro Bio Tech and his team for their collaboration regarding the use of "L2" laboratory of the Integrated and Urban Plant Pathology department for DNA extraction.

Funding: This study was conducted with the financial support of the Service Public of Wallonia (D°32-0343).

341 References

342 Al-Zeyara, S.A., Jarvis, B., Mackey, B.M., 2011. The inhibitory effect of natural microflora
343 of food on growth of *Listeria monocytogenes* in enrichment broths. Int. J. Food
344 Microbiol. 145, 98–105. https://doi.org/10.1016/j.ijfoodmicro.2010.11.036
345 Amézquita, A., Brashears, M.M., 2002. Competitive inhibition of *Listeria monocytogenes* in
346 ready-to-eat meat products by lactic acid bacteria. J. Food Prot. 65, 316–325.
347 https://doi.org/10.4315/0362-028X-65.2.316
348 Anang, D.M., Rusul, G., Bakar, J., Ling, F.H., 2007. Effects of lactic acid and lauricidin on
349 the survival of *Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli*
350 O157:H7 in chicken breast stored at 4°C. Food Control 18, 961–969.
351 https://doi.org/10.1016/j.foodcont.2006.05.015
352 Anonymous, 2003. Factors that influence microbial growth. Compr. Rev. Food Sci. Food Saf.
353 2, 21–32. https://doi.org/10.1111/j.1541-4337.2003.tb00048.x
354 Ariyapitipun, T., Mustapha, A., Clarke, A.D., 2000. Survival of *Listeria monocytogenes* Scott
355 A on vacuum-packaged raw beef treated with polylactic acid, lactic acid, and nisin. J.
356 Food Prot. 63, 131–136. https://doi.org/10.4315/0362-028X-63.1.131
357 Arqués, J.L., Rodríguez, E., Gaya, P., Medina, M., Nuñez, M., 2005. Effect of combinations
358 of high-pressure treatment and bacteriocin-producing lactic acid bacteria on the
359 survival of *Listeria monocytogenes* in raw milk cheese. Int. Dairy J. 15, 893–900.
360 https://doi.org/10.1016/j.idairyj.2004.07.020
361 Beaufort, A., Bergis, H., Lardeux, A.L., Lombard, B., 2014. EURL *Lm* technical guidance
362 document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat
363 foods.

364 Benner, R.A., 2014. Organisms of concern but not foodborne or confirmed foodborne:
365 spoilage microorganisms, in: Encyclopedia of Food Safety. Elsevier, pp. 245–250.
366 https://doi.org/10.1016/B978-0-12-378612-8.00169-4
367 Brandt, A.L., Castillo, A., Harris, K.B., Keeton, J.T., Hardin, M.D., Taylor, T.M., 2010.
368 Inhibition of *Listeria monocytogenes* by food antimicrobials applied singly and in
369 combination. J. Food Sci. 75, M557–M563. https://doi.org/10.1111/j.1750-
370 3841.2010.01843.x
371 Caplice, E., 1999. Food fermentations: role of microorganisms in food production and
372 preservation. Int. J. Food Microbiol. 50, 131–149. https://doi.org/10.1016/S0168-
373 1605(99)00082-3
374 Coenye, T., Vandamme, P., 2003. Diversity and significance of *Burkholderia* species
375 occupying diverse ecological niches. Environ. Microbiol. 5, 719–729.
376 https://doi.org/10.1046/j.1462-2920.2003.00471.x
377 Cogan, T.M., Barbosa, M., Beuvier, E., Bianchi-Salvadori, B., Cocconcelli, P.S., Fernandes,
378 I., Gomez, J., Gomez, R., Kalantzopoulos, G., Ledda, A., Medina, M., Rea, M.C.,
379 Rodriguez, E., 1997. Characterization of the lactic acid bacteria in artisanal dairy
380 products. J. Dairy Res. 64, 409–421. https://doi.org/10.1017/S0022029997002185
381 Commission Regulation, 2005. (EC) N° 2073/2005 of 15 November 2005 on microbiological
382 criteria for foodstuffs. Off. J. Eur. Union 338, 1–26.
383 Corless, C.E., Guiver, M., Borrow, R., Edwards-Jones, V., Kaczmarski, E.B., Fox, A.J., 2000.
384 Contamination and Sensitivity Issues with a Real-Time Universal 16S rRNA PCR. J.
385 Clin. Microbiol. 38, 1747–1752. https://doi.org/10.1128/JCM.38.5.1747-1752.2000
386 Delbes, C., Ali-Mandjee, L., Montel, M.-C., 2007. Monitoring bacterial communities in raw
387 milk and cheese by culture-dependent and -independent 16S rRNA gene-based

388 analyses. Appl. Environ. Microbiol. 73, 1882–1891.
389 https://doi.org/10.1128/AEM.01716-06
390 Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey,
391 D., Korsak, N., Daube, G., 2014. Microbiota characterization of a Belgian protected
392 designation of origin cheese, Herve cheese, using metagenomic analysis. J. Dairy Sci.
393 97, 6046–6056. https://doi.org/10.3168/jds.2014-8225
394 Dortu, C., Thonart, P., 2009. Les bactériocines des bactéries lactiques: caractéristiques et
395 intérêts pour la bioconservation des produits alimentaires/Bacteriocins from lactic acid
396 bacteria: interest for food products biopreservation. Biotechnol. Agron. Soc. Environ.
397 13, 143.
398 ElBalat, N., AbdElAal, S., Ayoub, M., Elsayed, M., 2014. Enumeration and characterization
399 of *Aeromonas* spp. isolated from milk and some dairy products in Sharkia
400 governorate, Egypt. Alex. J. Vet. Sci. 40, 52. https://doi.org/10.5455/ajvs.49073
401 El-Hajjaji, S., Gérard, A., De Laubier, J., Di Tanna, S., Lainé, A., Patz, V., Sindic, M., 2020.
402 Assessment of growth and survival of *Listeria monocytogenes* in raw milk butter by
403 durability tests. Int. J. Food Microbiol. 321, 108541.
404 https://doi.org/10.1016/j.ijfoodmicro.2020.108541
405 El-Hajjaji, S., Gérard, A., De Laubier, J., Di Tanna, S., Lainé, A., Patz, V., Sindic, M., 2019.
406 Overview of the local production process of raw milk butter in Wallonia (Belgium).
407 Int. J. Dairy Technol. https://doi.org/10.1111/1471-0307.12608
408 European Food Safety Authority and European Centre for Disease Prevention and Control
409 (EFSA and ECDC), 2019. The European Union one health 2018 zoonoses report.
410 EFSA J. 17. https://doi.org/10.2903/j.efsa.2019.5926
411 FASFC, 2019. Avis 11-2019 Potentiel de croissance de *Listeria monocytogenes* dans le beurre
412 de ferme au lait cru [WWW Document]. URL

http://www.afsca.be/comitescientifique/avis/2019/_documents/Avis11-2019_SciCom2018-17_listerialaitcrubeurre_000.pdf

Fernandez, P.S., George, S.M., Sills, C.C., Peck, M.W., 1997. Predictive model of the effect of CO2, pH, temperature and NaCl on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 37, 37–45.

Fricker, M., Skånseng, B., Rudi, K., Stessl, B., Ehling-Schulz, M., 2011. Shift from farm to dairy tank milk microbiota revealed by a polyphasic approach is independent from geographical origin. Int. J. Food Microbiol. 145, S24–S30. https://doi.org/10.1016/j.ijfoodmicro.2010.08.025

Garofalo, C., Bancalari, E., Milanović, V., Cardinali, F., Osimani, A., Sardaro, M.L.S., Bottari, B., Bernini, V., Aquilanti, L., Clementi, F., Neviani, E., Gatti, M., 2017. Study of the bacterial diversity of foods: PCR-DGGE versus LH-PCR. Int. J. Food Microbiol. 242, 24–36. https://doi.org/10.1016/j.ijfoodmicro.2016.11.008

Gérard, A., El-Hajjaji, S., Van Coillie, E., Bentaïb, A., Daube, G., Sindic, M., 2020. Determination of the growth potential of *Listeria monocytogenes* in various types of Belgian artisanal cheeses by challenge tests. Food Microbiol. 92, 103582. https://doi.org/10.1016/j.fm.2020.103582

Gnanamanickam, S.S. (Ed.), 2007. Plant-associated bacteria. Springer, Dordrecht.

Goerges, S., Aigner, U., Silakowski, B., Scherer, S., 2006. Inhibition of *Listeria monocytogenes* by food-borne yeasts. Appl. Environ. Microbiol. 72, 313–318. https://doi.org/10.1128/AEM.72.1.313-318.2006

Grahn, N., Olofsson, M., Ellnebo-Svedlund, K., Monstein, H.-J., Jonasson, J., 2003. Identification of mixed bacterial DNA contamination in broad-range PCR amplification of 16S rDNA V1 and V3 variable regions by pyrosequencing of cloned

amplicons. FEMS Microbiol. Lett. 219, 87–91. https://doi.org/10.1016/S0378-1097(02)01190-4

Guessas, B., Adjouj, F., Hadadji, M., Kihal, M., 2012. Isolation and identification of lactic acid bacteria from Dhan, a traditional butter and their major technological traits. World Appl. Sci. J. 17, 480–488.

Hayman, M.M., Kouassi, G.K., Anantheswaran, R.C., Floros, J.D., Knabel, S.J., 2008. Effect of water activity on inactivation of *Listeria monocytogenes* and lactate dehydrogenase during high pressure processing. Int. J. Food Microbiol. 124, 21–26. https://doi.org/10.1016/j.ijfoodmicro.2008.02.026

Hébraud, M., Potier, P., 1999. Cold shock response and low temperature adaptation in psychrotrophic bacteria. J. Mol. Microbiol. Biotechnol. 1, 211–219.

Holliday, S.L., Adler, B.B., Beuchat, L.R., 2003. Viability of *Salmonella*, *Escherichia coli* O157: H7, and *Listeria monocytogenes* in butter, yellow fat spreads, and margarine as affected by temperature and physical abuse. Food Microbiol. 20, 159–168.

Jayashree, S., Pushpanathan, M., Rajendhran, J., Gunasekaran, P., 2013. Microbial diversity and phylogeny analysis of buttermilk, a fermented milk product, employing 16S rRNA-based pyrosequencing. Food Biotechnol. 27, 213–221. https://doi.org/10.1080/08905436.2013.811084

Jofré, A., Garriga, M., Aymerich, T., Pérez Rodríguez, F., Valero, A., Carrasco, E., Bover Cid, S., 2016. Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready to eat (RTE) foods: activity 1, an extensive literature search and study selection with data extraction on *L. monocytogenes* in a wide range of RTE food. EFSA Support. Publ. 13. https://doi.org/10.2903/sp.efsa.2016.EN-1141

460 Kämpfer, P., Rosselló-Mora, R., Hermansson, M., Persson, F., Huber, B., Falsen, E., Busse,
461 H.-J., 2007. *Undibacterium pigrum* gen. nov., sp. nov., isolated from drinking water.
462 Int. J. Syst. Evol. Microbiol. 57, 1510–1515. https://doi.org/10.1099/ijs.0.64785-0
463 Kim, M., Wells, J.E., 2016. A Meta-analysis of bacterial diversity in the feces of cattle. Curr.
464 Microbiol. 72, 145–151. https://doi.org/10.1007/s00284-015-0931-6
465 Kim, S.-J., Moon, J.-Y., Weon, H.-Y., Hong, S.-B., Seok, S.-J., Kwon, S.-W., 2014.
466 *Undibacterium jejuense* sp. nov. and *Undibacterium seohonense* sp. nov., isolated
467 from soil and freshwater, respectively. Int. J. Syst. Evol. Microbiol. 64, 236–241.
468 https://doi.org/10.1099/ijs.0.056846-0
469 Koo, O.-K., Eggleton, M., O'Bryan, C.A., Crandall, P.G., Ricke, S.C., 2012. Antimicrobial
470 activity of lactic acid bacteria against *Listeria monocytogenes* on frankfurters
471 formulated with and without lactate/diacetate. Meat Sci. 92, 533–537.
472 https://doi.org/10.1016/j.meatsci.2012.05.023
473 Kuehn, J.S., Gorden, P.J., Munro, D., Rong, R., Dong, Q., Plummer, P.J., Wang, C., Phillips,
474 G.J., 2013. Bacterial community profiling of milk samples as a means to understand
475 culture-negative bovine clinical mastitis. PLoS ONE 8, e61959.
476 https://doi.org/10.1371/journal.pone.0061959
477 Lafarge, V., Ogier, J.-C., Girard, V., Maladen, V., Leveau, J.-Y., Gruss, A., Delacroix-
478 Buchet, A., 2004. Raw cow milk bacterial population shifts attributable to
479 refrigeration. Appl. Environ. Microbiol. 70, 5644–5650.
480 https://doi.org/10.1128/AEM.70.9.5644-5650.2004
481 Lin, C.-M., Moon, S.S., Doyle, M.P., McWatters, K.H., 2002. Inactivation of *Escherichia coli*
482 O157:H7, *Salmonella enterica* serotype Enteritidis, and *Listeria monocytogenes* on
483 lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat.
484 J. Food Prot. 65, 1215–1220. https://doi.org/10.4315/0362-028X-65.8.1215

485 Liu, W., Zheng, Y., Kwok, L.-Y., Sun, Z., Zhang, J., Guo, Z., Hou, Q., Menhe, B., Zhang, H.,
486 2015. High-throughput sequencing for the detection of the bacterial and fungal
487 diversity in Mongolian naturally fermented cow's milk in Russia. BMC Microbiol. 15,
488 45. https://doi.org/10.1186/s12866-015-0385-9
489 Magalhães, R., Mena, C., Ferreira, V., Silva, J., Almeida, G., Gibbs, P., Teixeira, P., 2014.
490 Bacteria: *Listeria monocytogenes*, in: Encyclopedia of Food Safety. Elsevier, pp. 450–
491 461. https://doi.org/10.1016/B978-0-12-378612-8.00101-3
492 Michelon, D., Leclercq, A., Garric, G., Guillier, L., Beaufort, A., Bergis, H., 2016. Growth
493 potential assessment of *Listeria* in milk fat products by challenge testing: growth
494 potential of *Listeria* in milk fat products. J. Food Saf. 36, 260–270.
495 https://doi.org/10.1111/jfs.12239
496 Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D.A., Desmasures, N.,
497 Berthier, F., 2014. Traditional cheeses: rich and diverse microbiota with associated
498 benefits. Int. J. Food Microbiol. 177, 136–154.
499 https://doi.org/10.1016/j.ijfoodmicro.2014.02.019
500 Moore, J.E., McILhatton, B., Shaw, A., Murphy, P.G., Elborn, J.S., 2001. Occurrence of
501 *Burkholderia cepacia* in foods and waters: clinical implications for patients with cystic
502 fibrosis. J. Food Prot. 64, 1076–1078. https://doi.org/10.4315/0362-028X-64.7.1076
503 Murdock, C.A., Cleveland, J., Matthews, K.R., Chikindas, M.L., 2007. The synergistic effect
504 of nisin and lactoferrin on the inhibition of *Listeria monocytogenes* and *Escherichia*
505 *coli* O157:H7. Lett. Appl. Microbiol. 44, 255–261. https://doi.org/10.1111/j.1472-
506 765X.2006.02076.x
507 Nyhan, L., Begley, M., Mutel, A., Qu, Y., Johnson, N., Callanan, M., 2018. Predicting the
508 combinatorial effects of water activity, pH and organic acids on *Listeria* growth in

media and complex food matrices. Food Microbiol. 74, 75–85. https://doi.org/10.1016/j.fm.2018.03.002

Oliveira, G.B. de, Favarin, L., Luchese, R.H., McIntosh, D., 2015. Psychrotrophic bacteria in milk: How much do we really know? Braz. J. Microbiol. 46, 313–321. https://doi.org/10.1590/S1517-838246220130963

Pérez-Rodríguez, F., Carrasco, E., Bover-Cid, S., Jofré, A., Valero, A., 2017. Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 2, a quantitative risk characterization on *L. monocytogenes* in RTE foods; starting from the retail stage. EFSA Support. Publ. 14. https://doi.org/10.2903/sp.efsa.2017.EN-1252

Perin, L.M., 2012. Intereference of storage temperatures in the development of mesophilic, psychrotrophic, lipolytic and proteolytic microbiota of raw milk. Semina Ciênc. Agrár. 33, 333–342. https://doi.org/10.5433/1679-0359.2012v33n1p333

Perin, L.M., Pereira, J.G., Bersot, L.S., Nero, L.A., 2019. The microbiology of raw milk, in: Raw Milk. Elsevier, pp. 45–64. https://doi.org/10.1016/B978-0-12-810530-6.00003-1

Phumudzo, T., Ronald, N., Khayalethu, N., Fhatuwani, M., 2013. Bacterial species identification getting easier. Afr. J. Biotechnol. 12, 5975–5982. https://doi.org/10.5897/AJB2013.12057

Potapov, V., Ong, J.L., 2017. Examining Sources of Error in PCR by Single-Molecule Sequencing. PLOS ONE 12, e0169774. https://doi.org/10.1371/journal.pone.0169774

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Quigley, L., O'Sullivan, O., Stanton, C., Beresford, T.P., Ross, R.P., Fitzgerald, G.F., Cotter, P.D., 2013. The complex microbiota of raw milk. FEMS Microbiol. Rev. 37, 664–698. https://doi.org/10.1111/1574-6976.12030

Raats, D., Offek, M., Minz, D., Halpern, M., 2011. Molecular analysis of bacterial communities in raw cow milk and the impact of refrigeration on its structure and dynamics. Food Microbiol. 28, 465–471. https://doi.org/10.1016/j.fm.2010.10.009

Saad, N.M., Amin, W.F., 2012. Isolation of *Burkholderia cepacia* complex from raw milk of different species of dairy animals in Assiut governorate 58, 4.

Salazar, J.K., Carstens, C.K., Ramachandran, P., Shazer, A.G., Narula, S.S., Reed, E., Ottesen, A., Schill, K.M., 2018. Metagenomics of pasteurized and unpasteurized gouda cheese using targeted 16S rDNA sequencing. BMC Microbiol. 18. https://doi.org/10.1186/s12866-018-1323-4

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12. https://doi.org/10.1186/s12915-014-0087-z

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09

Schvartzman, M.S., Belessi, C., Butler, F., Skandamis, P.N., Jordan, K.N., 2011. Effect of pH and water activity on the growth limits of *Listeria monocytogenes* in a cheese matrix at two contamination levels. J. Food Prot. 74, 1805–1813.

Shokralla, S., Spall, J.L., Gibson, J.F., Hajibabaei, M., 2012. Next-generation sequencing technologies for environmental DNA research. Mol. Ecol. 21, 1794–1805. https://doi.org/10.1111/j.1365-294X.2012.05538.x

Soomro, A.H., Tarik, M., Kiran, A., 2002. Role of lactic acid bacteria (LAB) in food preservation and human health – A review. Pak. J. Nutr. 1, 20–24. https://doi.org/10.3923/pjn.2002.20.24

Stiles, M.E., Holzapfel, W.H., 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol. 36, 1–29. https://doi.org/10.1016/S0168-1605(96)01233-0

Syromyatnikov, M.Y., Kokina, A.V., Solodskikh, S.A., Panevina, A.V., Popov, E.S., Popov, V.N., 2020. High-throughput 16S rRNA gene sequencing of butter microbiota reveals a variety of opportunistic pathogens. Foods 9, 608. https://doi.org/10.3390/foods9050608

Teixeira de Carvalho, A.A., Aparecida de Paula, R., Mantovani, H.C., Alencar de Moraes, C., 2006. Inhibition of *Listeria monocytogenes* by a lactic acid bacterium isolated from Italian salami. Food Microbiol. 23, 213–219. https://doi.org/10.1016/j.fm.2005.05.009

Voysey, P.A., Anslow, P.A., Bridgwater, K.J., Lavender, B., Watson, L., 2009. The effects of butter characteristics on the growth of *Listeria monocytogenes*. Int. J. Dairy Technol. 62, 326–330. https://doi.org/10.1111/j.1471-0307.2009.00505.x

Wang, C., Chang, T., Yang, H., Cui, M., 2015. Antibacterial mechanism of lactic acid on physiological and morphological properties of *Salmonella Enteritidis*, *Escherichia coli* and *Listeria monocytogenes*. Food Control 47, 231–236. https://doi.org/10.1016/j.foodcont.2014.06.034

Widyastuti, Y., Rohmatussolihat, Febrisiantosa, A., 2014. The role of lactic acid bacteria in milk fermentation. Food Nutr. Sci. 05, 435–442. https://doi.org/10.4236/fns.2014.54051

583 Yu, J., Mo, L., Pan, L., Yao, C., Ren, D., An, X., Tsogtgerel, T., Zhang, H., Liu, W., 2018.
584 Bacterial microbiota and metabolic character of traditional sour cream and butter in
585 Buryatia, Russia. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.02496
586 Yu, J., Wang, W.H., Menghe, B.L.G., Jiri, M.T., Wang, H.M., Liu, W.J., Bao, Q.H., Lu, Q.,
587 Zhang, J.C., Wang, F., Xu, H.Y., Sun, T.S., Zhang, H.P., 2011. Diversity of lactic acid
588 bacteria associated with traditional fermented dairy products in Mongolia. J. Dairy
589 Sci. 94, 3229–3241. https://doi.org/10.3168/jds.2010-3727
590 Yue, J.C., Clayton, M.K., 2005. A similarity measure based on species proportions. Commun.
591 Stat. - Theory Methods 34, 2123–2131. https://doi.org/10.1080/STA-200066418
592

593 Table 1a: pH and microbiological characteristics (averages ± standard deviations) of cream
594 samples during maturation

Cream samples	**Inoculation**	**Day of sampling**	**pH**	**Total microbial counts (log cfu/g)**	**LAB (log cfu/g)**	***L. monocytogenes* (log cfu/g)**
Cream_B1	Blank	D'0	6.77 ± 0.01	4.11 ± 1.05	3.88 ± 0.06	/
		D'1	6.80 ± 0.01	6.13 ± 0.05	4.65 ± 0.05	/
		D'2	6.78 ± 0.01	7.19 ± 0.02	4.47 ± 0.05	/
		D'3	6.76 ± 0.01	7.25 ± 0.04	5.13 ± 0.11	/
	Inoculated	D'0	NA	4.95 ± 0.04	4.29 ± 0.03	2.19 ± 0.14
		D'1	NA	6.27 ± 0.17	5.42 ± 0.30	2.80 ± 0.13
		D'2	NA	6.96 ± 0.10	5.31 ± 0.11	3.01 ± 0.16
		D'3	NA	7.25 ± 0.06	5.47 ± 0.23	3.75 ± 0.06
Cream_B2	Blank	D'0	6.75 ± 0.01	5.09 ± 0.08	3.93 ± 0.10	/
		D'1	6.69 ±	8.30 ±	8.03 ±	/

			0.01	0.00	0.47
		D’2	5.34 ±	8.22 ±	8.23 ±	/
			0.04	0.07	0.07	
		D’3	4.58 ±	7.88 ±	7.88 ±	/
			0.01	0.03	0.02	
	Inoculated	D’0	NA	5.14 ±	4.38 ±	1.75 ± 0.39
				0.15	0.12	
		D’1	NA	8.30 ±	8.30 ±	4.26 ± 0.08
				0.00	0.00	
		D’2	NA	7.90 ±	8.23 ±	5.00 ± 0.11
				0.05	0.08	
		D’3	NA	8.14 ±	8.00 ±	5.18 ± 0.04
				0.27	0.26	

595 B1: cream maturation at 4 °C, B2: cream maturation at 14 °C
596 D’0: cream before maturation, D’1: cream after 1 day of maturation, D’2: cream after 2 days
597 of maturation, D’3: cream after 3 days of maturation.
598 NA: Not Available.

599 Table 1b: pH, a_w and microbiological characteristics (averages ± standard deviations) of

600 butter samples during storage

Butter samples	Inoculation	Day of sampling	pH	a_w	Total microbial counts (log cfu/g)	LAB (log cfu/g)
Butter_B1	Blank	D0	6.81 ± 0.01	0.98 ± 0.00	5.46 ± 0.14	4.43 ± 0.37
		D7	5.60 ± 0.09	/	7.27 ± 0.04	5.82 ± 0.10
		D14	5.60 ± 0.03	/	7.11 ± 0.09	6.49 ± 0.05
		D30	5.39 ± 0.03	0.96 ± 0.00	6.72 ± 0.08	6.75 ± 0.22
	Inoculated	D0	NA	0.98 ± 0.00	5.48 ± 0.17	4.08 ± 0.00
		D7	NA	/	7.33 ± 0.16	5.75 ± 0.11
		D14	NA	/	7.25 ± 0.05	6.49 ± 0.08
		D30	NA	0.97 ± 0.01	6.57 ± 0.01	6.55 ± 0.07
Butter_B2	Blank	D0	4.75 ± 0.04	0.98 ± 0.00	7.31 ± 0.06	7.44 ± 0.23
		D7	4.58 ± 0.11	/	7.28 ± 0.06	7.22 ± 0.03
		D14	4.47 ± 0.03	/	6.97 ± 0.39	6.62 ± 0.02
		D30	4.52 ± 0.02	0.97 ± 0.00	6.21 ± 0.38	6.68 ± 0.16
	Inoculated	D0	NA	0.98 ± 0.00	6.96 ± 0.34	7.14 ± 0.47
		D7	NA	/	7.04 ± 0.33	7.20 ± 0.03
		D14	NA	/	6.24 ± 0.41	6.15 ± 0.36
		D30	NA	0.97 ± 0.00	4.38 ± 0.10	4.22 ± 0.03

601 B1: cream maturation at 4 °C, B2: cream maturation at 14 °C

602 D0: butter before storage, D7: butter after 7 days of storage, B14: butter after 14 days of

603 storage, D30: butter after 30 days of storage.

604 NA: Not Available

605 Table 2a: Richness and diversity indices (averages ± standard deviations) of cream samples

Sample	Inoculation	Day of sampling	Number of OTUs	Chao1 index	Shannon index	Inverse Simpson index
Cream_B1	Blank	D'0	228 ± 123	1255 ± 489	4.21 ± 1.34	32 ± 33
		D'1	290 ± 18	1495 ± 75	5.10 ± 0.14	78 ± 19
		D'2	297 ± 2	1643 ± 46	5.22 ± 0.02	109 ± 14
		D'3	306 ± 17	2111 ± 205	5.29 ± 0.08	135 ± 13
	Inoculated	D'0	307 ± 10	1536 ± 100	5.10 ± 0.07	51 ± 7
		D'1	293 ± 28	1624 ± 353	5.17 ± 0.22	106 ± 38
		D'2	279 ± 26	1713 ± 386	5.11 ± 0.18	107 ± 31
		D'3	244 ± 16	985 ± 763	4.88 ± 0.12	76 ± 11
Cream_B2	Blank	D'0	300 ± 25	1625 ± 172	5.01 ± 0.21	47 ± 16
		D'1	272 ± 72	2474 ± 1494	4.95 ± 0.47	87 ± 55
		D'2	102 ± 8	763 ± 58	2.86 ± 0.09	8 ± 1
		D'3	82 ± 7	840 ± 9	2.55 ± 0.09	7 ± 0
	Inoculated	D'0	289 ± 39	1551 ± 358	4.92 ± 0.41	48 ± 22
		D'1	194 ± 25	1358 ± 263	4.20 ± 0.29	28 ± 8
		D'2	73 ± 12	774 ± 175	2.16 ± 0.15	4 ± 0
		D'3	85 ± 10	901 ± 120	2.29 ± 0.12	4 ± 0

606 B1: cream maturation at 4 °C, B2: cream maturation at 14 °C
607 D'0: cream before maturation, D'1: cream after 1 day of maturation, D'2: cream after 2 days
608 of maturation, D'3: cream after 3 days of maturation.

609 Table 2b: Richness and diversity indices (averages ± standard deviations) of butter samples

Sample	Inoculation	Day of sampling	Number of OTUs	Chao1 index	Shannon index	Inverse Simpson index
Butter_B1	Blank	D0	239 ± 57	1338 ± 224	4.17 ± 0.64	25 ± 12
		D7	329 ± 4	2134 ± 348	4.96 ± 0.06	50 ± 9
		D14	287 ± 3	2433 ± 334	4.60 ± 0.15	31 ± 12
		D30	278 ± 39	1706 ± 210	4.58 ± 0.18	31 ± 2
	Inoculated	D0	251 ± 17	1764 ± 487	4.38 ± 0.23	28 ± 12
		D7	312 ± 18	2250 ± 713	4.87 ± 0.09	52 ± 6
		D14	299 ± 26	1723 ± 132	4.79 ± 0.25	52 ± 17
		D30	278 ± 18	2328 ± 621	4.61 ± 0.11	36 ± 2
Butter_B2	Blank	D0	116 ± 8	1144 ± 341	2.72 ± 0.05	7 ± 0
		D7	107 ± 17	608 ± 69	2.58 ± 0.17	6 ± 1
		D14	103 ± 3	678 ± 171	2.57 ± 0.04	6 ± 0
		D30	94 ± 17	829 ± 398	2.30 ± 0.18	5 ± 1
	Inoculated	D0	94 ± 4	677 ± 29	2.14 ± 0.03	4 ± 0
		D7	120 ± 27	932 ± 449	2.42 ± 0.30	4 ± 1
		D14	77 ± 3	550 ± 270	1.93 ± 0.08	4 ± 0
		D30	75 ± 8	1629 ± 383	1.77 ± 0.08	3 ± 0

610 B1: cream maturation at 4 °C, B2: cream maturation at 14 °C
611 D0: butter before storage, D7: butter after 7 days of storage, B14: butter after 14 days of
612 storage, D30: butter after 30 days of storage.

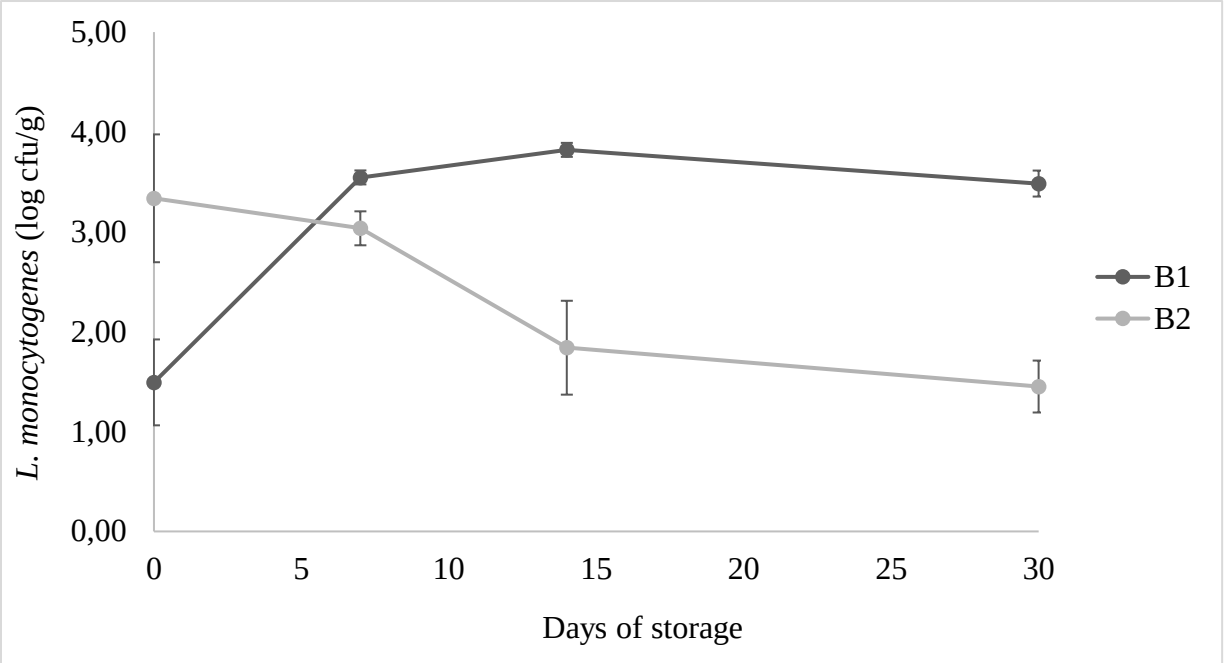


Figure 1: Evolution of levels of *L. monocytogenes* in the two batches of raw milk butter during storage.
B1: First batch with cream matured at 4 °C, B2: Second batch with cream matured at 14 °C
Three samples were analyzed each time. Each point represents the mean value of the three measurements and the vertical line represents the standard deviation

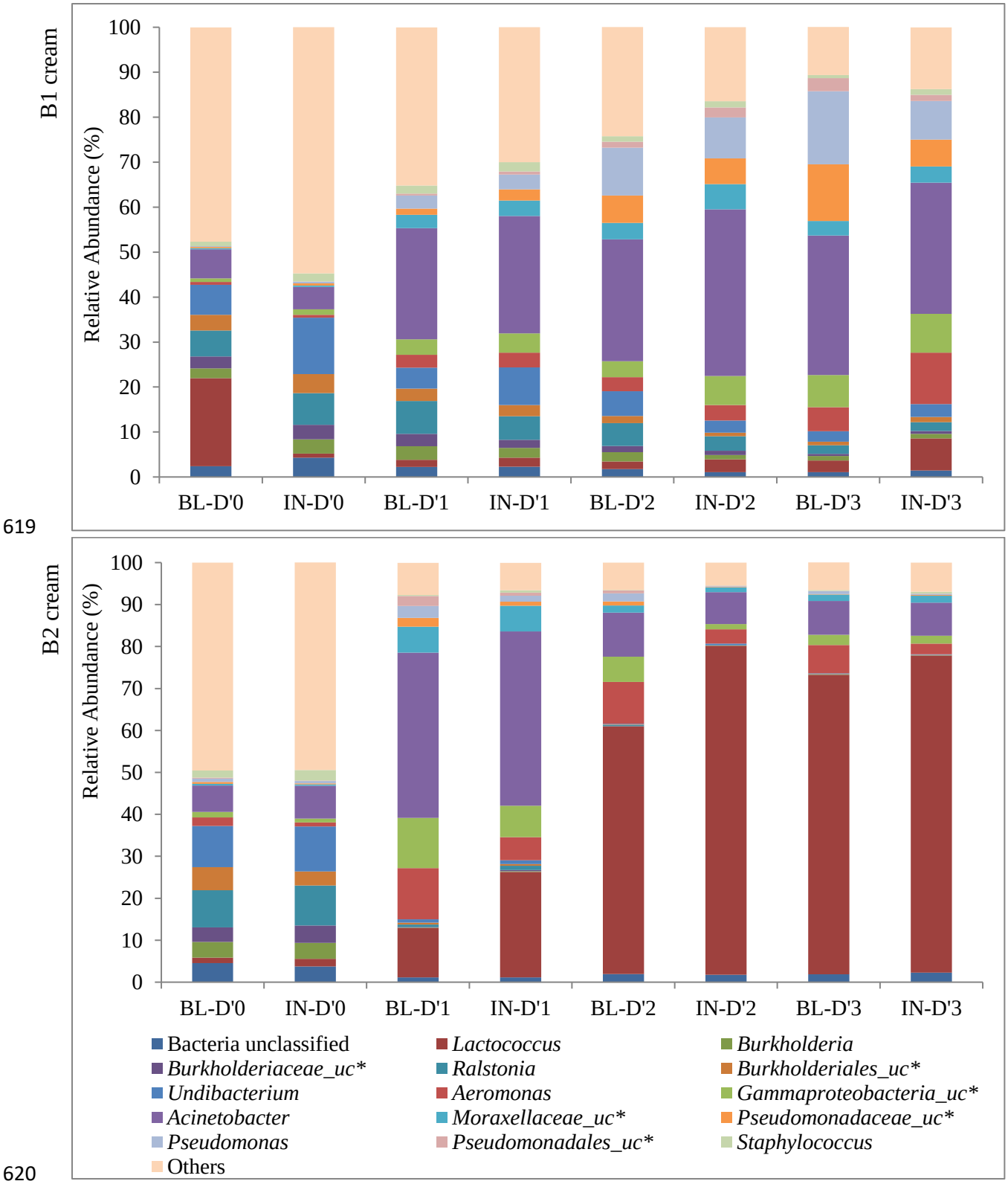


Figure 2: Bacterial microbiota distribution of the two batches of cream samples (B1: cream maturation at 4 °C, B2: cream maturation at 14 °C) depending on day of sampling (D'0, D'1, D'2 and D'3) and the presence or not of *L. monocytogenes* (BL: blank samples, IN: inoculated samples).

* uc: unclassified bacteria

Burkholderiaceae (family): unclassified genera of the family *Burkholderiaceae;*

Burkholderiales (order): unclassified family (other than *Burkholderiaceae*) belonging to *Burkholderiales;*
Moraxellaceae (family): unclassified genera of the family *Moraxellaceae;*
Pseudomonadaceae (family): unclassified genera of the family *Pseudomonadaceae;*
Pseudomonadales (order): unclassified family (other than *Moraxellaceae* and *Pseudomonadaceae*) belonging to *Pseudomonadales;*
Gammaproteobacteria (class): unclassified order (other than *Pseudomonadales*) of the class *Gammaproteobacteria*

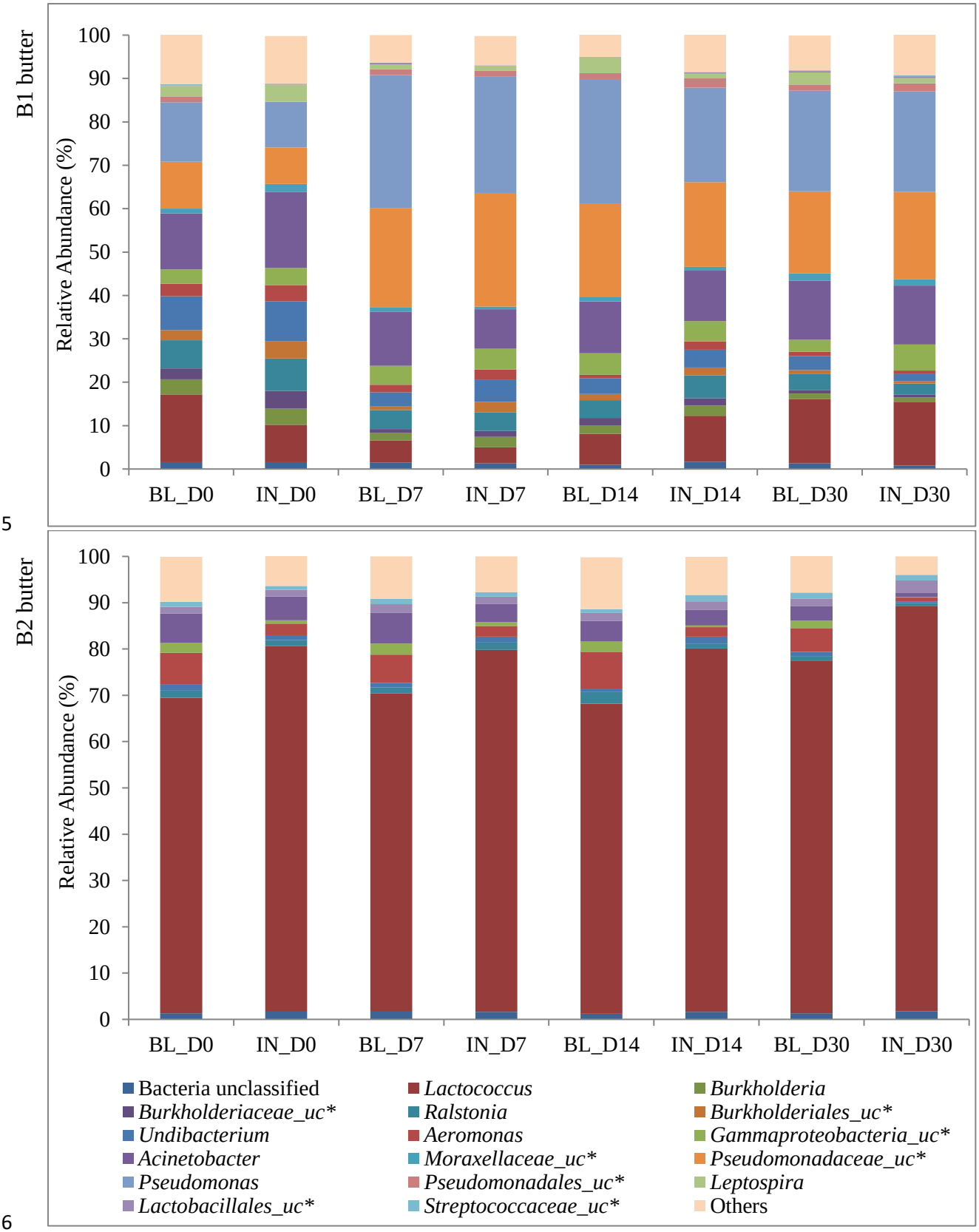
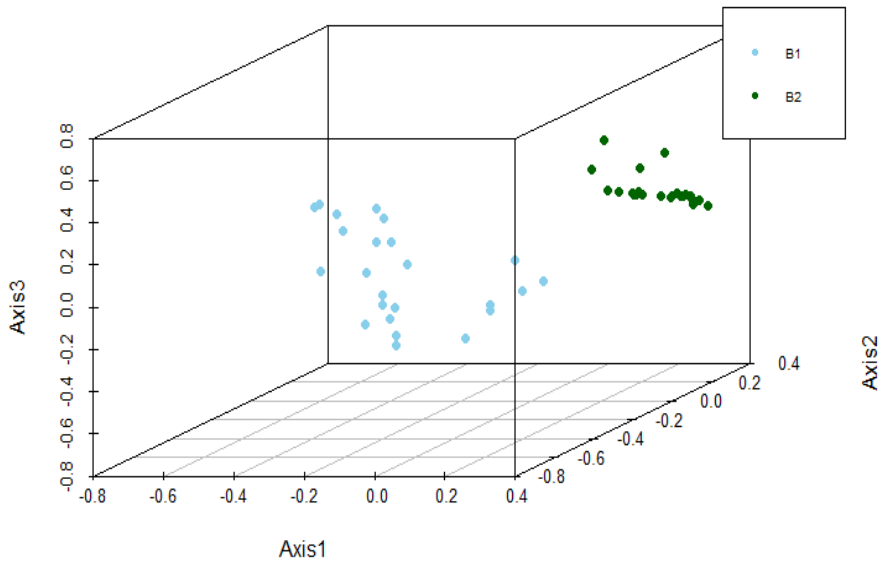


Figure 3: Bacterial microbiota distribution of the two batches of butter samples (B1: cream maturation at 4 °C, B2: cream maturation at 14 °C) depending on storage period (D0, D7, D14 and D30) and the presence or not of *L. monocytogenes* (BL: blank samples, IN: inoculated samples).

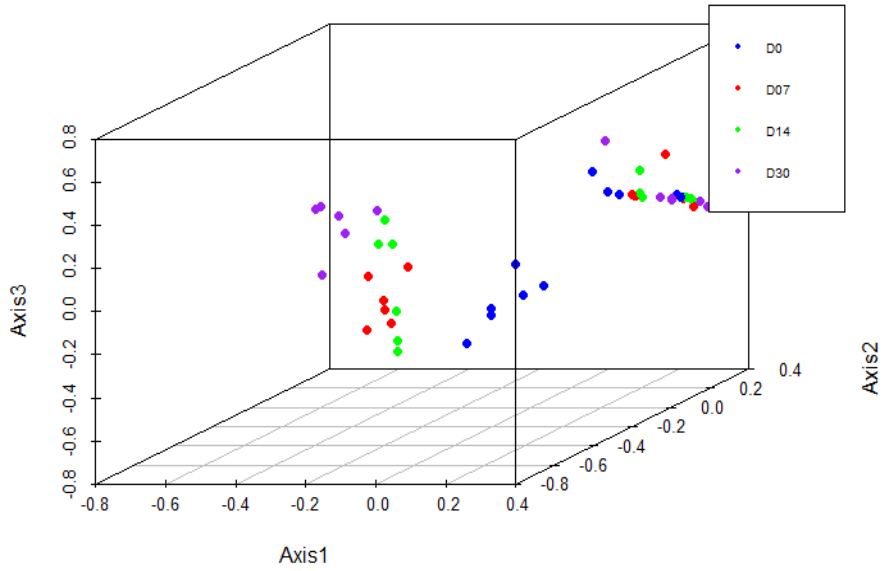
* uc: unclassified bacteria
Burkholderiaceae (family): unclassified genera of the family *Burkholderiaceae;*
Burkholderiales (order): unclassified family (other than *Burkholderiaceae*) belonging to *Burkholderiales;*
Moraxellaceae (family): unclassified genera of the family *Moraxellaceae;*
Pseudomonadaceae (family): unclassified genera of the family *Pseudomonadaceae;*
Pseudomonadales (order): unclassified family (other than *Moraxellaceae* and *Pseudomonadaceae*) belonging to *Pseudomonadales;*
Gammaproteobacteria (class): unclassified order (other than *Pseudomonadales*) of the class *Gammaproteobacteria;*
Streptococcaceae (family): unclassified genera of *Streptococcaceae;*
Lactobacillales (order): unclassified family (other than *Streptococcaceae*) of *Lactobacillales*

A)



653

B)



654

655 Figure 4: NMDS plot of butter samples generated via Yue & Clayton distance matrix,
656 depending on cream maturation (A) and storage period (B). B1: cream maturation at 4 °C, B2:
657 cream maturation at 14 °C, D0: butter before storage, D7: butter after 7 days of storage, D14:
658 butter after 14 days of storage, D30: butter after 30 days of storage