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COMPARISON OF RAW COW MILK MICROBIOTA IN TWO MILKING SYSTEMS: A FIELD STUDY

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Abstract

This study investigated the effect of different milking systems on the milk composition and microbial community of raw milk in a commercial dairy farm. Raw milk samples from conventional milking system (CMS) and automatic milking system (AMS) were collected and the microbiota on each was assessed by 16S rRNA gene sequencing. Results showed that the acetone (P = 0.031) and β-hydroxybutyrate (P≤0.001) levels in the raw milk of the AMS group were increased compared with the CMS group. Principal component analysis, unweighted and weighted principal coordinates analysis, and heat map of microbial community composition in the raw milk showed a clear separation between two groups. AMS increased the abundance of the genera *Acinetobacter* (FDR = 0.004) and *Staphylococcus* (FDR = 0.004) in the raw milk compared with the CMS group. In contrast, the abundance of the genera *Pseudomonas* (FDR = 0.028), *Lactococcus* (FDR = 0.015), *Sphingobacterium* (FDR = 0.004), *Brevundimonas* (FDR = 0.005), and *Chryseobacterium* (FDR = 0.042) in the raw milk was reduced in the AMS group compared with the CMS group. The abundance of the genera *Acinetobacter* and *Staphylococcus* in the raw milk was positively correlated with the β-hydroxybutyrate, acetone, free fatty acid, citric acid, and urea nitrogen levels. Furthermore, the abundance of the genus *Acinetobacter* in the raw milk was negatively correlated with the somatic cell counts. The study demonstrates that the introduction of AMS in the dairy farm can regulate microbiota composition in the raw milk and this modification may exert an effect on reducing the somatic cell counts in the raw milk.

Key words: automatic milking system, microbiota, raw milk, conventional milking system

The raw cow milk microbiota is known to harbor complex microbial communities with a significant impact on the hygienic, sensory, texture, and flavor quality of milk (Wouters et al., 2002). The diversity of raw milk microbiota also affects the safety and pricing of subsequent development of dairy products (Quigley et al., 2013). Several microorganisms involving milk quality have been identified in raw cow milk (von Neubeck et al., 2015; Porcellato et al., 2018). In addition, mastitis, an inflammation of the mammary gland caused by infectious and environmental pathogens, is the most important disease in dairy cows. The somatic cell counts in milk are positively associated with the incidence of mastitis in dairy cows. Several studies have demonstrated that the specific microbial communities in raw milk are correlated with somatic cell counts (Oikonomou et al., 2014; Rodrigues et al., 2017; Tong et al., 2019). Therefore, controlling the microbial composition of raw milk is particularly important to the dairy industry in order to prevent mastitis and maintain the health of dairy cows.

The automatic milking system (AMS) or milking robot is increasingly implemented in modern dairy farms. Compared with the regular twice per day milking of conventional milking system (CMS) management, AMS can improve cow comfort and health, increase milk production, and reduce labor for milking (Jacobs and Siegford, 2012; Hansen, 2015; Tse et al., 2018). Furthermore, the risk of udder contamination during milking is expected to be minimized in AMS compared with the CMS. Therefore, the microbiota of raw milk is supposedly different between AMS and CMS. However, information on the raw cow milk microbiota of AMS is limited compared to that of raw milk collected from CMS.

It has been reported that the somatic cell counts are increased after milking with the AMS (Kruip et al., 2002; Hovinen and Pyörälä, 2011). The introduction of AMS in dairy farms results in a decrease in milk quality compared with the CMS (Klungel et al., 2000). The risk of clinical mastitis in pasture-fed cows managed in an AMS is increased (Hammer et al., 2012). It has been demonstrated dysbiosis in the milk microbiome can occur during the progression of mastitis by increasing opportunistic pathogenic bacteria and reducing healthy milk commensal bacteria (Hoque et al., 2019). The distribution of dominant and subdominant microorganisms in raw milk can change the milk composition, thereby affecting the flavor, taste, and texture of raw milk (Quigley et al., 2013). The raw milk microbiota can be affected from a variety of sources, including milking equipment. Whether altera-

tion of raw milk microbiota by different milking systems correlates with somatic cell count and milk composition is still rarely investigated.

Therefore, we hypothesize that raw cow milk microbiota can be differentially regulated between CMS and AMS in the dairy farm and specific microbial communities modulated by different milking systems are associated with milk composition. The objective of the present study was to compare the microbiota profiles of raw milk collected from different milking systems, and to explore the correlations between the milk composition and microbiota.

Material and methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee of National Ilan University.

Sample collection

A total of 18 individual milk samples (9 samples from CMS group and 9 samples from AMS group) from Holstein-Friesian cows with similar average days in milk were collected in November 2020 at a commercial farm (Tainan, Taiwan), which operated CMS (herringbone stall) and AMS (Astronaut A4 robotic milking system, Lely, Maassluis, The Netherlands) for management of 100 lactating dairy cows (20 cows for CMS and 80 cows for AMS, respectively). All cows were housed in a free stall barn. Cows in the CMS group were fed total mixed ration (TMR) and milked twice daily. Milk samples from the CMS group were collected at 9:00 and 20:00 h. Cows in the AMS group were fed partial mixed ration (PMR) in combination with pelleted concentrates in the milking stall for encouraging cows to come to the stall frequently for milking. Milk samples from the AMS group were collected after cows were milked voluntarily (twice or three times a day). The minimum and maximum temperatures on the day of sampling were 22.1 and 28.3°C. All dairy cows were considered healthy and no antibiotic treatments were administered during the sampling period. All samples after collection were immediately refrigerated on ice and transported to Milk Testing Laboratory (Livestock Research Institute, Miaoli, Taiwan).

Raw milk composition analysis

Milk samples were analyzed in the Milk Testing Laboratory (Livestock Research Institute, Miaoli, Taiwan). Milk quality traits (fat, protein, lactose, urea nitrogen, citric acid, total solids, solids-not-fat, casein, free fatty acid, total saturated fatty acid, total unsaturated fatty acid, acetone, and β-hydroxybutyrate) were assessed using Milko-Scan FT+ (Foss Electric A/S, Hillerød, Denmark). Somatic cell counts were determined using a Fossomatic FC flow cytometry somatic cell counter (Foss Electric A/S, Hillerød, Denmark).

16S rRNA gene sequencing and data analysis

For the extraction of microbial DNA, a bacterial pellet was prepared from 200 μL of each milk sample after enzymatic lysis and centrifugation. DNA was then purified using the magnetic bead-based method using the MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems, Foster City, CA). The yield and purity of the extracted DNA were assessed with a Quibt 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA). For 16S rRNA gene sequencing, the polymerase chain reaction (PCR) amplification using primers targeting the V3 and V4 region of the 16S rRNA genes (forward: 5′-CCTACGGGNGGCWGCAG-3′; reverse: 5′-GACTACHVGGGTATCTAATCC-3′) was employed. The polymerase chain reaction temperature cycling conditions were as follows: initiation at 95°C for 3 min and followed by 30 cycles of 95°C for 30 s, 57°C for 30 s, 72°C for 30 s, and a final elongation of 72°C for 5 min. The products were purified using the QIAquick Gel Extraction kit (QIAGEN, Germantown, MD, USA). Sequencing libraries were generated using TruSeq Nano DNA Library Prep kits (Illumina, San Diego, CA, USA) and sequenced on an Illumina Miseq platform by a commercial company (Genomics, New Taipei City, Taiwan). After sequencing, the quality of raw reads was checked with FastQC software (version 0.11.5). Raw reads were processed following QIIME software (version 1.9.0) and primers were trimmed using Cutadapt software (version 1.16). Low-quality reads with a quality score < 20 and sequences below length thresholds were removed. The sequences of 16S rRNA gene were then clustered into the operational taxonomic unit (OTU) with a 97% similarity threshold in Mothur software (version 1.39.5) and UCHIME software (version 4.2). For minimizing the effects of random sequencing errors, the original OTUs were modified and clustered by removing OTUs containing less than 10 sequences. The modified OTUs were used as a basis for subsequent analysis. The rarefaction curves were used for sequence count normalization in QIIME software. A Venn diagram was created using Venn diagram (version 1.6.17) to compare the number of common and unique bacteria (genus-level) between groups. Taxonomic analysis, alpha diversity (species richness and species evenness), and advanced analysis (principal component analysis, unweighted UniFrac principal coordinate analysis, and weighted UniFrac principal coordinate analysis) were performed in QIIME software. The correlation matrix visualization was created using R package corrplot (version 0.84).

Statistical analysis

The SAS package (Version 9.4; SAS Institute, Cary, NC, USA) was used to analyze the significance of all results. Student's *t* test was used for intergroup comparison. A P value of less than 0.05 was statistically significant and a P value between 0.05 and 0.1 was considered a trend. The principal component analysis and principal coordinate analysis were executed using UniFrac distan-

ces coupled with standard multivariate statistics in QIIME software. For microbiota analysis, Benjamini-Hochberg false discovery rate (BH-FDR) correction (FDR P value) was used to adjust for multiple testing. The correlations between milk bacteria abundance and milk composition were measured by Pearson correlation coefficient. The significance of the correlation FDR P value <0.05 was the threshold to define significant correlations. Weak correlation is in the range of 0.1 to 0.3, moderate correlation from 0.3 to 0.6, and strong correlation from 0.6 to 1.0.

Results

Effect of different milking systems on milk composition and microbial diversity

The result of milk composition between two milking systems is shown in Table 1. No significant difference was found in major milk composition except acetone and β-hydroxybutyrate levels. The acetone (P = 0.031) and β-hydroxybutyrate (P≤0.001) levels in raw milk in the AMS group were significantly increased compared with the CMS group. A trend of increased citric acid levels in raw milk was observed in the AMS group compared with the CMS group (P = 0.088). After stringent quality trimming of raw data, the averages of high-quality reads from the raw milk of CMS and AMS groups were 29,500 (minimal high-quality reads: 23,429) and 22,782 (minimal high-quality reads: 18,698), respectively. The average OTUs from the raw milk of CMS and AMS groups were 3,594 and 2,167, respectively. The results of alpha diversity of microbiota between two milking systems are shown in Table 2. The bacterial species richness (Chao1 and Fisher alpha estimator) in raw milk was significantly reduced in the AMS group compared with the CMS group (FDR = 0.004 and FDR = 0.007). Similarly, the AMS significantly decreased the bacterial species evenness (Shannon and Enspie estimator) of raw milk compared with the CMS group (FDR = 0.004 and FDR = 0.013). The Venn diagram illustrated an overlap (87 bacteria, core) that was shared by two of the plotted groups (Figure 1). In total, 318 and 19 unique bacteria were found in the CMS and AMS groups, respectively. Principal component analysis (PCA) revealed statistically significant discrimination between the two groups (FDR = 0.004, Figure 2 A). Principal coordinate analysis (PCoA) based on qualitative traits (unweighted UniFrac distances) and quantitative traits (weighted UniFrac distances) also indicated that the microbiota of raw milk samples was clearly separated between the two groups (FDR = 0.001 and FDR = 0.002, Figure 2 B and Figure 2 C).

Table 1. Milk composition of raw milk between two milking systems

	CMS[1]	AMS[1]	SEM	P value
Somatic cell count (× 10^4 cells/mL)	26.17	11.60	4.72	0.126
Fat (%)	3.47	2.99	0.20	0.243
Protein (%)	3.41	3.21	0.10	0.329
Lactose (%)	4.92	4.93	0.05	0.853
Urea nitrogen (mg/dL)	12.24	12.96	0.57	0.552
Citric acid (mg/dL)	138.89	153.78	4.36	0.088
Total solids (%)	12.50	11.83	0.25	0.197
Solids-not-fat (%)	9.02	8.84	0.11	0.435
Casein (%)	2.63	2.49	0.08	0.394
Free fatty acid (mmol/100 g fat)	1.57	2.72	0.59	0.347
Total saturated fatty acid (%)	2.44	2.02	0.17	0.230
Total unsaturated fatty acid (%)	0.78	0.68	0.05	0.341
Acetone (μmol/L)	2.00 a	71.00 b	0.016	0.031
β-hydroxybutyrate (μmol/L)	18.00 a	93.00 b	0.012	< 0.001

[1]CMS = conventional milking system; AMS = automatic milking system.
a, b – different letters within a row are numerically different (P≤0.05).

Table 2. Microbial α-diversity of raw milk between two milking systems

	CMS[1]	AMS[1]	SEM	FDR P value[1]
Chao1	129.9 a	41.6 b	16.00	0.004
Fisher alpha	15.6 a	4.4 b	2.15	0.007
Shannon	3.5 a	2.5 b	0.16	0.004
Enspie	5.6 a	4.0 b	0.34	0.013

[1]CMS = conventional milking system; AMS = automatic milking system; FDR P value = Benjamini-Hochberg false discovery rate (FDR) – corrected P value.
a, b – different letters within a row are numerically different (P≤0.05).

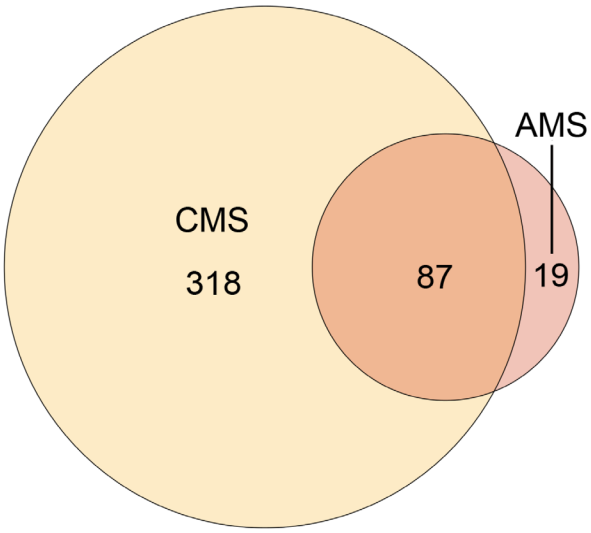


Figure 1. Venn diagram illustrating the bacteria (genus-level) distribution in the raw milk between two milking system groups. Each ellipse represents one group. The overlapping regions between the ellipses represent the bacterial number that is shared between the conventional milking system (CMS) and automatic milking system (AMS) (n = 9). The value of each region represents the number of bacteria corresponding to the region

Effects of different milking systems on the bacteria taxonomic composition and community structure in the raw milk

The results of bacterial taxonomy in the raw milk between two milking systems are shown in Table 3. At the phylum level, the abundance of the phylum Bacteroidetes (FDR = 0.005) in the raw milk was decreased in the AMS group compared with the CMS group. At the class level, the abundance of the classes Bacteroidia (FDR = 0.003) and Alphaproteobacteria (FDR = 0.004) in the raw milk were reduced in the AMS group compared with the CMS group. At the order level, AMS increased the abundance of the order Bacillales (FDR = 0.003) in the raw milk compared with the CMS group. The abundance of the orders Lactobacillales (FDR = 0.006), Betaproteobacteriales (FDR = 0.021), Sphingobacteriales (FDR = 0.004), Flavobacteriales (FDR = 0.021), Caulobacterales (FDR = 0.005), and Rhizobiales (FDR = 0.029) in the raw milk was lower in the AMS group compared with the CMS group. At the family level, the abundance of the families Moraxellaceae (FDR = 0.004), Staphylococcaceae (FDR = 0.005), and Family XII (FDR = 0.004) in the raw milk was higher in the AMS group compared with the CMS group. The abundance of the families Pseudomonadaceae (FDR = 0.021), Streptococcaceae (FDR = 0.021), Burkholderiaceae (FDR = 0.021), Sphingobacteriaceae (FDR = 0.004), Caulobacteraceae (FDR = 0.005), and Weeksellaceae (FDR = 0.038) in the raw milk was lower in the AMS group compared with the CMS group. At the genus level, AMS increased the abundance of the genera *Acinetobacter* (FDR = 0.004), *Staphylococcus* (FDR = 0.004), and *Exiguobacterium* (FDR = 0.004) in the raw milk compared with the CMS group. The abundance of the genera *Pseudomonas* (FDR = 0.028), *Lactococcus* (FDR = 0.015), *Sphingobacterium* (FDR = 0.004), *Brevundimonas* (FDR = 0.005), and *Chryseobacterium* (FDR = 0.042) in the raw milk was reduced in the AMS group compared with the CMS group. A heat map of the 35 most abundant genera in the raw milk is presented in Figure 3. The results showed that AMS resulted in unique bacterial community clusters in the raw milk compared with the CMS group, such as the genera *Acinetobacter*, *Staphylococcus*, *Exiguobacterium*, and *Bacillales_unclassified*. In contrast, some bacterial community clusters were specifically increased in the CMS group, such as genera *Corynebacterium 1*, *Flavobacterium*, *Achromobacter*, *Ignavigranum*, *Lactobacillus*, *Jeotgalibaca*, *Solibacillus*, and *Sphingomonas*.

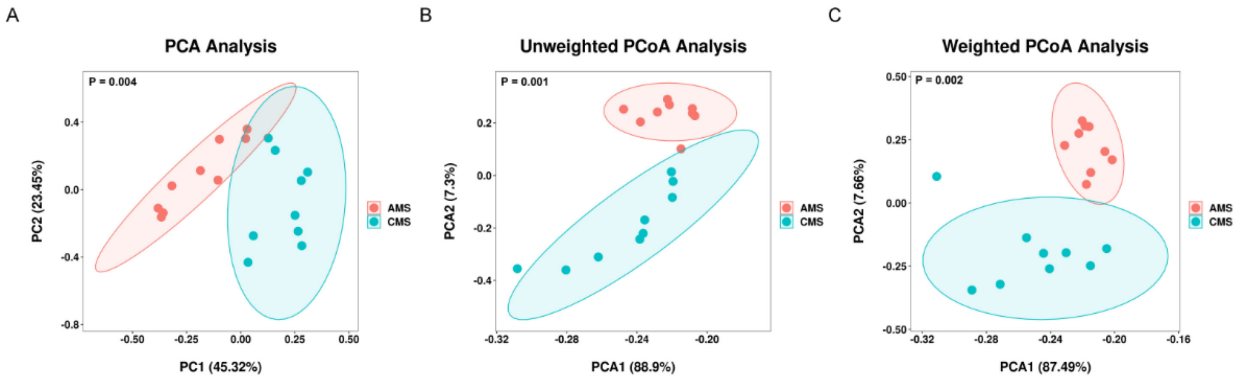


Figure 2. Principal component analysis and principal coordinate analysis of the bacterial communities in the raw milk. (A) Principal component analysis plots of the raw milk of conventional milking system (CMS) and automatic milking system (AMS) (n = 9). Principal coordinate analysis plots of unweighted UniFrac distances (B) and weighted UniFrac distances (C) of the bacterial communities from CMS and AMS (n = 9)

Table 3. Bacterial taxonomy within the raw milk between two milking systems

	Relative abundance (%)		SEM	FDR P value[1]
	CMS[1]	AMS[1]		
Phylum				
Proteobacteria	65.5	60.7	4.62	0.739
Firmicutes	23.9	38.8	4.78	0.185
Bacteroidetes	8.3 a	0.3 b	1.20	0.005
Actinobacteria	2.1	0.2	0.46	0.126
Class				
Gammaproteobacteria	59.4	60.5	4.51	0.901
Bacilli	22.2	38.8	4.58	0.136
Bacteroidia	8.3 a	0.3 b	1.20	0.003
Alphaproteobacteria	6.1 a	0.1 b	1.09	0.004
Actinobacteria	1.9	0.2	0.43	0.053
Order				
Pseudomonadales	39.0	56.3	4.56	0.054
Bacillales	2.3 a	35.1 b	4.88	0.003
Lactobacillales	19.9 a	3.7 b	3.08	0.006
Betaproteobacteriales	11.4 a	0.2 b	2.41	0.021
Enterobacteriales	6.4	3.8	2.03	0.526
Sphingobacteriales	4.3 a	0.2 b	0.68	0.004
Flavobacteriales	3.7 a	0.1 b	0.76	0.021
Caulobacterales	3.5 a	0.1 b	0.62	0.005
Xanthomonadales	2.5	0.2	0.62	0.076
Rhizobiales	2.1 a	0.1 b	0.48	0.029
Family				
Moraxellaceae	18.7 a	51.0 b	5.08	0.004
Pseudomonadaceae	20.2 a	5.3 b	3.23	0.021
Staphylococcaceae	1.8 a	22.2 b	3.60	0.005
Streptococcaceae	16.1 a	3.4 b	2.62	0.021
Burkholderiaceae	11.4 a	0.2 b	2.41	0.021
Family XII	0.3 a	12.5 b	2.12	0.004
Enterobacteriaceae	6.4	3.8	2.03	0.526
Sphingobacteriaceae	4.3 a	0.2 b	0.68	0.004
Caulobacteraceae	3.5 a	0.1 b	0.62	0.005
Weeksellaceae	3.4 a	0.1 b	0.76	0.038
Genus				
Acinetobacter	18.0 a	51.0 b	5.10	0.004
Pseudomonas	20.2 a	5.3 b	3.24	0.028
Staphylococcus	0.5 a	22.2 b	3.66	0.004
Lactococcus	10.9 a	3.3 b	1.50	0.015
Exiguobacterium	0.3 a	12.5 b	2.12	0.004
Delftia	8.5	0.1	2.35	0.090
Streptococcus	5.2	< 0.1	2.54	0.345
Enterobacteriaceae_unclassified	4.1	3.7	0.86	0.417
Sphingobacterium	3.7 a	0.2 b	0.66	0.004
Serratia	3.5	< 0.1	1.80	0.345
Brevundimonas	3.3 a	0.1 b	0.62	0.005
Chryseobacterium	2.4 a	0.1 b	0.76	0.042
Stenotrophomonas	1.8	0.2	0.62	0.090
Rhizobiaceae_unclassified	1.3	< 0.1	0.45	0.102
Achromobacter	1.2	< 0.1	0.56	0.254

[1]CMS = conventional milking system; AMS = automatic milking system. FDR P value = Benjamini-Hochberg false discovery rate (FDR) – corrected P value.

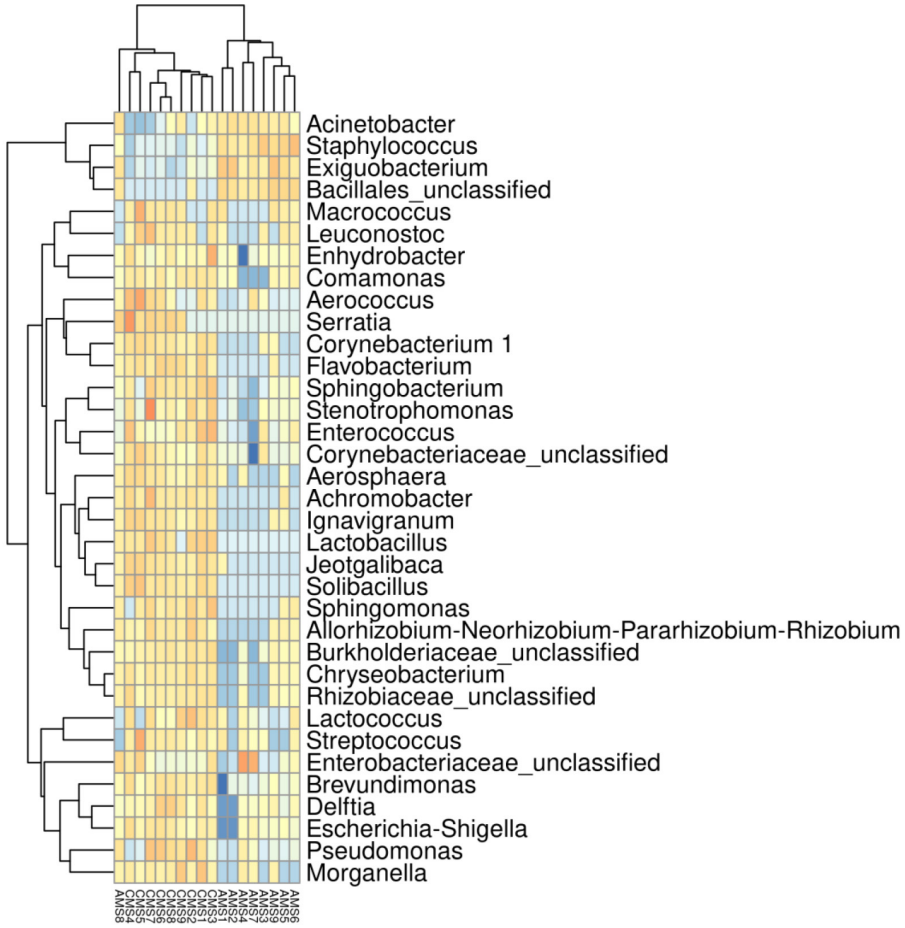


Figure 3. Species abundance heat map showing normalized values of dominant 35 genera of two milking systems. Samples from conventional milking system (CMS) or automatic milking system (AMS) is plotted on the X-axis (n = 9), and the Y-axis represents the genus

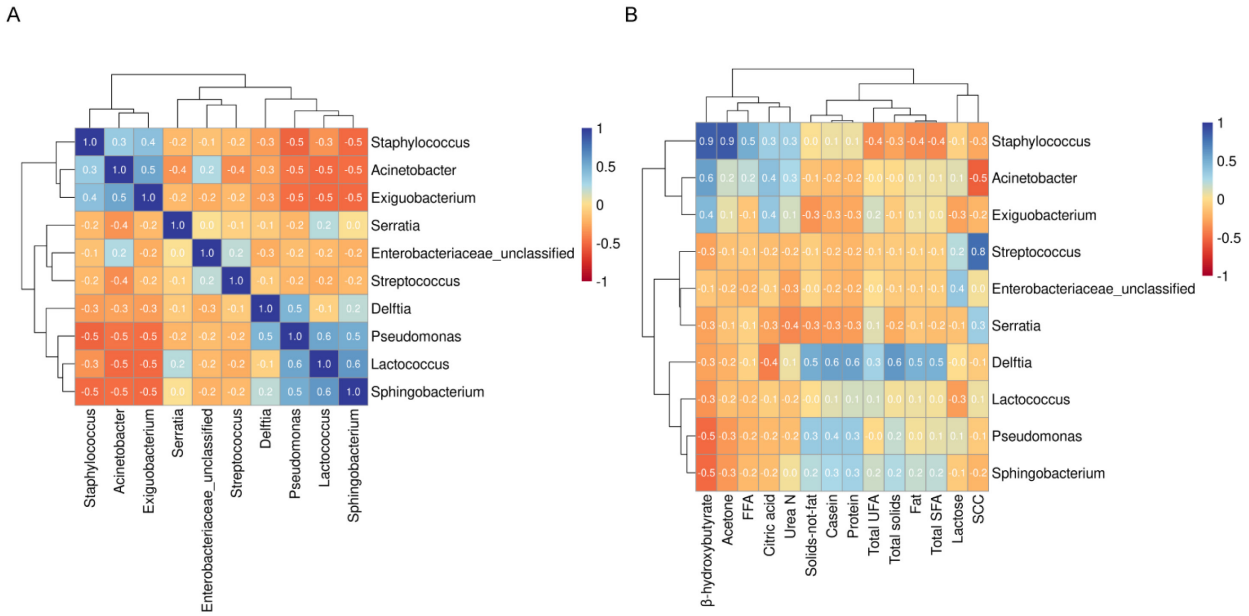


Figure 4. Association analysis between the average bacterial abundance and milk composition. (A) Relationship among the dominant 10 genera. (B) Relationship between the dominant 10 genera and milk composition. Positive correlations are displayed in blue, while negative correlations are displayed in red. The values from +1 to -1 indicate the strength of the correlation

Relationships between the average abundances of the bacterial genera and milk composition

The abundance of the genera *Sphingobacterium*, *Lactococcus*, and *Pseudomonas* were positively correlated with each other (Figure 4 A). In contrast, the abundance of the genera *Sphingobacterium*, *Lactococcus*, and *Pseudomonas* were negatively correlated with the average abundance of the genera *Staphylococcus*, *Acinetobacter*, and *Exiguobacterium* (Figure 4 A). The abundance of the genera *Staphylococcus*, *Acinetobacter*, and *Exiguobacterium* were positively correlated with each other (Figure 4 A). The average abundance of the genera *Staphylococcus* and *Acinetobacter* were positively associated with β-hydroxybutyrate, acetone, free fatty acid, citric acid, and urea nitrogen levels in the raw milk (Figure 4 B). The abundance of the genera *Delftia* and *Sphingobacterium* were positively associated with solids-not-fat, casein, protein, total unsaturated fatty acid, total solids, fat, and total saturated fatty acid levels in the raw milk (Figure 4 B). The abundance of the genus *Pseudomonas* was positively associated with solids-not-fat, casein, protein, and total solids levels in the raw milk (Figure 4 B). The abundance of the genus *Acinetobacter* was negatively associated with somatic cell counts, whereas the abundance of the genus *Streptococcus* was positively associated with somatic cell counts in the raw milk (Figure 4 B).

Discussion

It has been reported that the introduction of AMS in the dairy farm does not alter the fat and protein levels of milk, but significantly increases free fatty acid levels compared with the CMS (Klungel et al., 2000). Here, there was no significant difference in fat and protein levels of raw milk between CMS and AMS group, which is in agreement with a previous study (Klungel et al., 2000). However, no significant difference in free fatty acid levels of raw milk was observed between CMS and AMS groups in the present study, which contradicts the previous study (Klungel et al., 2000). Studies have shown inconsistent results regarding the effect of different milking systems on the somatic cell counts of milk (Klungel et al., 2000; Berglund et al., 2002; Kruip et al., 2002; Hovinen and Pyörälä, 2011). There are no differences in somatic cell counts of milk between the CMS and AMS groups (Klungel et al., 2000; Abeni et al., 2008). However, the somatic cell counts are increased after milking with the AMS (Kruip et al., 2002; Hovinen and Pyörälä, 2011). In contrast, the introduction of AMS significantly decreases the somatic cell counts of milk (Ipema and de Koning, 1997; Berglund et al., 2002). Here, there was no significant difference in somatic cell counts of raw milk between the CMS and AMS group. This finding is in agreement with the results of Abeni et al. (2008), who observed that CMS and AMS in the same farm with similar environment and management indicated no differences in somatic cell counts of milk. Interestingly, the acetone and β-hydroxybutyrate levels in the raw milk were increased in the AMS group in the present study, indicating that fatty acid metabolism and ketone body production were regulated in cows managed in an AMS. It has been demonstrated that the acetone and β-hydroxybutyrate levels in raw milk are positively correlated with acetone and β-hydroxybutyrate levels in the blood (Enjalbert et al., 2001). The elevation of blood β-hydroxybutyrate concentration above 1,200 μmol/L is considered as subclinical ketosis in dairy cows. Although the introduction of AMS increased the β-hydroxybutyrate levels in the raw milk in the present study, the level of milk β-hydroxybutyrate in the AMS group (93 μmol/L) was still far below 1,200 μmol/L. Whether the introduction of AMS in the dairy farm has a risk for subclinical ketosis in cows remains to be investigated.

It has been demonstrated that short milking intervals can flush bacteria from the udder more often, whereas long milking intervals provide bacteria to colonize the udder after milking (Bramley et al., 1981). For most of the cows, milking frequency is increased in AMS compared with the CMS (Hogeveen et al., 2001). The unique OTUs and bacterial species richness and evenness were reduced in the raw milk of the AMS group in the present study, demonstrating that the introduction of AMS can reduce the bacterial abundance and diversity of raw milk. The results of principal component analysis and principal coordinate analysis further indicate the microbial community in the raw milk of the AMS group was re-shaped. Here, the genus *Acinetobacter* is dominant in the raw milk of the AMS group, whereas the genus *Pseudomonas* is abundant in the raw milk of the CMS group. The genera *Acinetobacter* and *Pseudomonas* belong to psychrotrophic spoilage bacteria, which flourish during cold storage (Quigley et al., 2013). The abundance of the genus *Acinetobacter* in raw cow milk is positively correlated with the standard plate count (Rodrigues et al., 2017). The *Acinetobacter* strains isolated from raw milk exhibit antibiotic resistance (Gurung et al., 2013). In humans, the genus *Acinetobacter* is prevalent in the milk of the healthy group compared with the mastitic group (Patel et al., 2017). The genus *Pseudomonas* can cause severe clinical mastitis in dairy cows and milk spoilage (Kirk and Bartlett, 1984; Ercolini et al., 2009). The abundance of the genus *Acinetobacter* was inversely correlated with the abundance of the genus *Pseudomonas* in the raw milk, implying that genus *Acinetobacter* (51%) may control the population of genus *Pseudomonas* (5.3%) in the raw milk of the AMS group. Furthermore, the correlation result demonstrated that the abundance of the genus *Acinetobacter* was negatively associated (–0.5) with the somatic cell counts of raw milk. Taken together, these results indicate that the introduction of AMS in the dairy farm reduces the microbial diversity and increases the abundance of the genus *Acinetobacter* in raw cow milk. The abundance of the genus *Acinetobacter* in raw cow milk may have an impact on somatic cell counts.

Previous studies have demonstrated that somatic cell counts in raw cow milk are not correlated with the abundance of genus *Staphylococcus* (Oikonomou et al., 2014; Rodrigues et al., 2017). Here, there was a weak negative correlation (–0.3) between the abundance of the genus *Staphylococcus* and somatic cell counts in raw milk. The discrepancy among the studies may be due to the sampling population. The somatic cell counts range from health to clinical mastitis in the previous studies (Oikonomou et al., 2014; Rodrigues et al., 2017), whereas average somatic cell counts in CMS and AMS groups in the present study are 26.2×10^4 and 11.6×10^4 cells/mL, respectively. The genus *Staphylococcus* is one of the pathogens capable of causing mastitis in dairy cows, particularly *Staphylococcus aureus* and coagulase-negative staphylococci (Dufour et al., 2012). The abundance of the genus *Staphylococcus* in the raw milk was increased in the AMS group in the present study. In addition, a strong positive correlation (0.8) between the abundance of the genus *Staphylococcus* and the acetone and β-hydroxybutyrate levels in raw milk was found. The acetone and β-hydroxybutyrate levels in raw milk in the AMS group were also higher than in the CMS group. It has been reported that the elevated serum β-hydroxybutyrate levels predispose the cows to mastitis (Jánosi et al., 2003). However, based on somatic cell count results, the cows in the AMS group are not considered as subclinical mastitis although the abundance of the genus *Staphylococcus* and β-hydroxybutyrate levels in raw milk is high. Therefore, the effect of increased abundance of the genus *Staphylococcus* in raw milk of the AMS group on cow health still remains to be elucidated in the future.

The genus *Lactococcus*, a class of lactic acid bacteria, is naturally present in raw milk and cheese (Gaya et al., 1999). *Lactococcus* species, such as *Lactococcus lactis*, exhibit antibacterial activity against *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus* species through the production of antimicrobial peptides (Alegría et al., 2010; Ortolani et al., 2010; Perin et al., 2012). In the present study, the abundance of the genus *Lactococcus* in the raw milk was reduced in the AMS group, whereas the abundance of the genus *Staphylococcus* was increased. Furthermore, a weak negative correlation (–0.3) between the abundance of the genera *Lactococcus* and *Staphylococcus* were observed. These findings partially support the results of Perin et al. (2012), who demonstrated that *Lactococcus* species have an impact on the growth of *Staphylococcus* species. Since the *Lactococcus* species are able to ferment lactose to lactate, thereby decreasing the pH value in the raw milk (Fortina et al., 2009). Here, the abundance of the genus *Lactococcus* was also negatively correlated (–0.3) with the lactose levels in the raw milk. These results further confirm the relationship between the abundance of the genera *Lactococcus* and *Staphylococcus* or lactose levels in the raw milk.

The raw cow milk microbiota is highly associated with milk quality due to microbial enzymatic activity (Quigley et al., 2013). Microbial proteases and lipases can reduce the nutrient and economic value of milk by the hydrolysis of protein and lipid during the milk processes (Quigley et al., 2013). The psychrotrophic bacteria are able to cause a 10% loss of milk fats and proteins, particularly genera *Acinetobacter* and *Pseudomonas* (Hantsis-Zacharov and Halpern, 2007). It has been demonstrated that proteolytic and lipolytic activities of genus *Acinetobacter* contribute to the spoilage of raw milk (Hantsis-Zacharov and Halpern, 2007). In the present study, the abundance of genus *Acinetobacter* was not strongly associated with major milk quality parameters (protein, fat, lactose, solids-not-fat, and total solids), but moderately negatively correlated with somatic cell counts (–0.5) and positively correlated with β-hydroxybutyrate levels (0.6). Interestingly, the abundance of the genus *Pseudomonas* was weakly positively correlated with protein (0.3), casein (0.4), and solids-not-fat (0.3). This result contradicts the findings of another study, notably research published in Hantsis-Zacharov and Halpern (2007). In addition, it has been reported that the genus *Pseudomonas* is not the major bacteria responsible for the degradation of milk properties (Li et al., 2018). Therefore, the impact of genus *Pseudomonas* on milk quality still needs to be confirmed. The genus *Delftia* has been reported as a member of the raw cow milk microbiota and shows high proteolytic activity (Hantsis-Zacharov and Halpern, 2007; Oikonomou et al., 2014). In this study, the abundance of the genus *Delftia* was moderately positively correlated with several milk quality parameters (protein, fat, solids-not-fat, and total solids). This is the first report demonstrating a correlation between the abundance of the genus *Delftia* and milk quality. In addition to milk microbiota, it has been reported that gut-associated microbes may transfer from rumen to mammary gland, thereby affecting milk microbiota (Ted et al., 2014). The phylum Firmicutes is a common bacterial community in the rumen of lactating dairy cows (Tong et al., 2018). These species were also found in the milk microbiota of two milking systems in the present study. This finding is in agreement with the results of Tong et al. (2019), who also observed that Firmicutes bacteria was presented in raw milk. The abundance of the genus *Streptococcus* (belonging to Firmicutes) strongly positively correlated with somatic cell counts (0.8) in raw cow milk. This finding is in agreement with the results of Zanardi et al. (2014), who also observed that the abundance of the genus *Streptococcus* is associated with high somatic cell counts in raw cow milk and prevalence of subclinical mastitis. The abundance of the genus *Exiguobacterium* of the phylum Firmicutes was weakly negatively correlated with lactose (–0.3), protein (–0.3), casein (–0.3), and solids-not-fat (–0.3). Similarly, the abundance of the genus *Staphylococcus* of the phylum Firmicutes was moderately negatively associated with total saturated fatty acid (–0.4), fat (–0.4), and total unsaturated fatty acid (–0.4). Taken together, genera *Pseudomonas* and *Delftia* in raw cow milk may have a positive effect on milk quality. Raw milk microbiota

may partially originate from the entero-mammary pathway, particularly phylum Firmicutes. These bacteria (*Streptococcus*, *Exiguobacterium*, and *Staphylococcus*) may have an adverse effect on milk quality. Raw milk microbiota and gut microbiota may interact with each other and affect milk quality. Whether gut microbiota is also altered between two milking systems and has a direct impact on milk quality remains to be investigated in the future.

The microbiota in raw cow milk has been shown to vary by geographic region and season (Kim et al., 2017; Nguyen et al., 2020). For instance, the families Methylobacteriaceae and Moraxellaceae are prevalent in raw cow milk of cool season, whereas the families Staphylococcaceae, Bacillaceae, Ruminococcaceae, and Veillonellaceae are abundant in hot season (Nguyen et al., 2020). The abundance of the genera *Corynebacterium*, *Pediococcus*, *Macrococcus*, and *Acinetobacter* in the raw milk vary in different geographical locations (Kim et al., 2017). In the present study, it is the limitation that all raw milk samples were collected in November and at the same farm. Whether the introduction of AMS in the dairy farm in different geographic regions and the seasons also cause a similar effect on the raw cow milk microbiota remains to be elucidated. Thus, further research is required to clarify the effect of geographical and seasonal differences on raw cow milk microbiota between CMS and AMS.

Conclusions

These findings demonstrated that the introduction of AMS in the dairy farm can modulate the raw milk composition and bacterial community. The modification of milk microbiota by AMS is associated with the somatic cell counts and milk composition. The milking process is completely different between CMS and AMS, such as milking intervals, teat cleaning, and procedures for teat dipping. The difference in the milking process can affect raw milk microbiota directly. The current findings provide novel insights into the effect of AMS on the raw milk microbiota and the correlation between the bacterial community and milk composition.

References

Abeni F., Terzano M.G., Speroni M., Migliorati L., Capelletti M., Calza F., Bianchi L., Pirlo G. (2008). Evaluation of milk enzymes and electrolytes, plasma metabolites, and oxidative status in twin cows milked in an automatic milking system or twice daily in a conventional milking parlor. J. Dairy Sci., 91: 3372–3384.

Alegría A., Delgado S., Roces C., López B., Mayo B. (2010). Bacteriocins produced by wild *Lactococcus lactis* strains isolated from traditional, starter-free cheeses made of raw milk. Int. J. Food Microbiol., 143: 61–66.

Berglund I., Pettersson G., Svennersten-Sjaunja K. (2002). Automatic milking: Effects on somatic cell count and teat end-quality. Livest. Prod. Sci., 78: 115–124.

Bramley A.J., Godinho K.S., Grindal R.J. (1981). Evidence of penetration of the bovine teat duct by *Escherichia coli* in the interval between milkings. J. Dairy Res., 48: 379: 386.

Dufour S., Dohoo I.R., Barkema H.W., Descôteaux L., Devries T.J., Reyher K.K., Roy J.P., Scholl D.T. (2012). Manageable risk factors associated with the lactational incidence, elimination, and prevalence of *Staphylococcus aureus* intramammary infections in dairy cows. J. Dairy Sci., 95: 1283–1300.

Enjalbert F., Nicot M.C., Bayourthe C., Moncoulon R. (2001). Ketone bodies in milk and blood of dairy cows: relationship between concentrations and utilization for detection of subclinical ketosis. J. Dairy Sci., 84: 583–589.

Ercolini D., Russo F., Ferrocino I., Villani F. (2009). Molecular identification of mesophilic and psychrotrophic bacteria from raw cow's milk. Food Microbiol., 26: 228–231.

Fortina M.G., Ricci G., Borgo F. (2009). A study of lactose metabolism in *Lactococcus garvieae* reveals a genetic marker for distinguishing between dairy and fish biotypes. J. Food Prot., 72: 1248–1254.

Gaya P., Babín M., Medina M., Nuñez M. (1999). Diversity among lactococci isolated from ewes' raw milk and cheese. J. Appl. Microbiol., 87: 849–855.

Gurung M., Nam H.M., Tamang M.D., Chae M.H., Jang G.C., Jung S.C., Lim S.K. (2013). Prevalence and antimicrobial susceptibility of *Acinetobacter* from raw bulk tank milk in Korea. J. Dairy Sci., 96: 1997–2002.

Hammer J.F., Morton J.M., Kerrisk K.L. (2012). Quarter-milking-, quarter-, udder- and lactation-level risk factors and indicators for clinical mastitis during lactation in pasture-fed dairy cows managed in an automatic milking system. Aust. Vet. J., 90: 167–174.

Hansen B.G. (2015). Robotic milking-farmer experiences and adoption rate in Jaeren, Norway. J. Rural Stud., 41: 109–117.

Hantsis-Zacharov E., Halpern. M. (2007). Culturable psychrotrophic bacterial communities in raw milk and their proteolytic and lipolytic traits. Appl. Environ. Microbiol., 73: 7162–7168.

Hogeveen H., Ouweltjes W., de Koning C.J.A.M., Stelwagen K. (2001). Milking interval, milk production and milk flow-rate in an automatic milking system. Livest. Prod. Sci., 72: 157–167.

Hoque M.N., Istiaq A., Clement R.A., Sultana M., Crandall K.A., Siddiki A.Z., Hossain M.A. (2019). Metagenomic deep sequencing reveals association of microbiome signature with functional biases in bovine mastitis. Sci. Rep., 9: 1–14.

Hovinen M., Pyörälä S. (2011). Invited review: udder health of dairy cows in automatic milking. J. Dairy Sci., 94: 547–562.

Ipema B., de Koning K. (1997). Melken mit dem Melkroboter Milchpraxis, 35: 72–73.

Jacobs J., Siegford J. (2012). Invited review: The impact of automatic milking systems on dairy cow management, behaviour, health and welfare. J. Dairy Sci., 95: 2227–2247.

Jánosi S., Kulcsár M., Kóródi P., Kátai L., Reiczigel J., Dieleman S.J., Nikolic J.A., Sályi G., Ribiczey-Szabó P., Huszenicza G. (2003). Energy imbalance related predisposition to mastitis in group-fed high-producing postpartum dairy cows. Acta Vet. Hung., 51: 409–424.

Kim I.S., Hur Y.K., Kim E.J., Ahn Y.T., Kim J.G., Choi Y.J., Huh C.S. (2017). Comparative analysis of the microbial communities in raw milk produced in different regions of Korea. Asian-Australas. J. Anim. Sci., 30: 1643–1650.

Kirk J.H., Bartlett P.C. (1984). Nonclinical *Pseudomonas aeruginosa* mastitis in a dairy herd. J. Am. Vet. Med. Assoc., 184: 671–673.

Klungel G.H., Slaghuis B.A., Hogeveen H. (2000). The effect of the introduction of automatic milking systems on milk quality. J. Dairy Sci., 83: 1998–2003.

Kruip T.A., Morice H., Robert M., Ouweltjes W. (2002). Robotic milking and its effect on fertility and cell counts. J. Dairy Sci., 85: 2576–2781.

Li N., Wang Y., You C., Ren J., Chen W., Zheng H., Liu Z. (2018). Variation in raw milk microbiota throughout 12 months and the impact of weather conditions. Sci. Rep., 8: 2371.

Nguyen Q.D., Tsuruta T., Nishino N. (2020). Examination of milk microbiota, fecal microbiota, and blood metabolites of Jersey cows in cool and hot seasons. Anim. Sci. J., 91: e13441.

Oikonomou G., Bicalho M.L., Meira E., Rossi R.E., Foditsch C., Machado V.S., Teixeira A.G., Santisteban C., Schukken Y.H., Bicalho R.C. (2014). Microbiota of cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters. PLoS One, 9: e85904.

Ortolani M.B., Moraes P.M., Perin L.M., Viçosa G.N., Carvalho K.G., Silva Júnior A., Nero L.A. (2010). Molecular identification of naturally occurring bacteriocinogenic and bacteriocinogenic-like lactic acid bacteria in raw milk and soft cheese. J. Dairy Sci., 93: 2880–2886.

Patel S.H., Vaidya Y.H., Patel R.J., Pandit R.J., Joshi C.G., Kunjadiya A.P. (2017). Culture independent assessment of human milk microbial community in lactational mastitis. Sci. Rep., 7: 7804.

Perin L.M., Moraes P.M., Viçosa G.N., Silva Júnior A., Nero L.A. (2012). Identification of bacteriocinogenic *Lactococcus* isolates from raw milk and cheese capable of producing nisin A and nisin Z. Int. Dairy J., 25: 46–51.

Porcellato D., Aspholm M., Skeie S.B., Monshaugen M., Brendehaug J., Mellegard H. (2018). Microbial diversity of consumption milk during processing and storage. Int. J. Food Microbiol., 266: 21–30.

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.

Rodrigues M.X., Lima S.F., Canniatti-Brazaca S.G., Bicalho R.C. (2017). The microbiome of bulk tank milk: characterization and associations with somatic cell count and bacterial count. J. Dairy Sci., 100: 2536–2552.

Ted J., Christophe L., Braegger C.P., Florence R., Christophe C. (2014). Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environ. Microbiol., 16: 2891–2904.

Tong J., Zhang H., Yang D., Zhang Y., Xiong B., Jiang L. (2018). Illumina sequencing analysis of the ruminal microbiota in high-yield and low-yield lactating dairy cows. PLoS One, 13: e0198225.

Tong J., Zhang H., Zhang Y., Xiong B., Jiang L. (2019). Microbiome and metabolome analyses of milk from dairy cows with subclinical *Streptococcus agalactiae* mastitis – potential biomarkers. Front. Microbiol., 10: 2547.

Tse C., Barkema H.W., DeVries T.J., Rushen J., Pajor E.A. (2018). Impact of automatic milking systems on dairy cattle producers' reports of milking labour management, milk production and milk quality. Animal, 12: 2649–2656.

von Neubeck M., Baur C., Krewinkel M., Stoeckel M., Kranz B., Stressler T., Fischer L., Hinrichs J., Scherer S., Wenning M. (2015). Biodiversity of refrigerated raw milk microbiota and their enzymatic spoilage potential. Int. J. Food Microbiol., 211: 57–65.

Wouters J.T.M., Ayad E.H.E., Hugenholtz J., Smit G. (2002). Microbes from raw milk for fermented dairy products. Int. Dairy J., 12: 91–109.

Zanardi G., Caminiti A., Delle Donne G., Moroni P., Santi A., Galletti G., Tamba M., Bolzoni G., Bertocchi L. (2014). Short communication: comparing real-time PCR and bacteriological cultures for *Streptococcus agalactiae* and *Staphylococcus aureus* in bulk-tank milk samples. J. Dairy Sci., 97: 5592–5598.

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