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Effect of post-milking treatment on teat skin and milk microbial diversity of dairy cows

I. Verdier-Metz[1,*], P. Pradel[2], F. Monsallier[3] and M.C. Montel[1]

[1]NRA-UR545 Fromagères, 20 côte de Reyne, F-15000 Aurillac (France)
[2]INRA-UE1296 Monts d'Auvergne, La Borie, F-15190 Marcenat (France)
[3]Chambre d'Agriculture du Cantal, 28 boulevard Monthyon, F-15200 Mauriac (France)
*e-mail: iverdier@clermont.inra.fr

Abstract. Microbial diversity is one of the specificities of traditional raw milk cheeses. It depends on both milk microbiota and microbial dynamics during manufacturing and ripening. The microbial composition of milk is not clearly understood yet; it depends on direct and indirect sources which vary according to herd management (feed, herd size, lactation rank, etc.), farm environments (litter, dung) and milking management (udder microbial community). The surface of cow teats has been described as a source of microorganisms for milk. Thus, it can be hypothesized that practices modifying the teat skin microbiota could be a lever to increase qualitatively and quantitatively the microbial diversity in milk. To check this hypothesis, the composition of microbial species of the teats of dairy cows that received three post-milking treatments (iodine, glycerol, no products) over the lactation was compared. Teat microbiota was also compared to the microbial composition of the corresponding milk. Teat microbiota was highly dominated by ripening bacteria whereas that of milk was well balanced between the different microbial groups. Forty-three microbial genera were identified on the surface of the teat against 34 in milk: 20 genera were common. The post-milking treatment affected mainly the count of ripening bacteria of teat surface whereas it had no effect on milk microbiota. The count of other microbial populations was not modified whatever the treatment.

Keywords. Microbial diversity – Microbial flow – Milk – Teat surface.

Effet du post-trempage sur la diversité microbienne de la peau des trayons et du lait de vaches laitières

Résumé. *La diversité microbienne est une des spécificités des fromages traditionnels au lait cru. Elle dépend du microbiote du lait et de la dynamique microbienne au cours de la transformation fromagère et de l'affinage. La composition microbienne du lait n'est pas encore clairement comprise. Elle dépend de sources directes et indirectes dépendantes de la gestion du troupeau, de l'environnement et de la traite. Les trayons des vaches ont été décrits comme une source de micro-organismes pour le lait. Il a donc été émis l'hypothèse que les pratiques susceptibles de modifier le microbiote des trayons peuvent être un levier d'action pour favoriser la diversité microbienne du lait. Pour vérifier cette hypothèse, la composition des espèces microbiennes des trayons de vaches laitières ayant reçu trois traitements de post-traite sur l'ensemble de la lactation a été comparée. Elle a aussi été comparée à celle des laits correspondants. Le microbiote des trayons était dominé par les bactéries d'affinage alors que celui du lait était bien équilibré entre bactéries d'affinage, bactéries lactiques, bactéries Gram- et levures-moisissures. Quarante-trois genres microbiens ont été identifiés sur le trayon contre 34 dans le lait : 20 genres étaient communs au lait et aux trayons. Le post-trempage influe sur les bactéries d'affinage du trayon alors qu'il n'a eu aucun effet sur celle du lait. Les niveaux des autres populations n'ont pas été modifiés.*

Mots-clés. *Diversité microbienne – Flux microbien – Lait – Surface des trayons.*

I – Introduction

Traditional cheeses are mainly produced with raw milk in the south of Europe in particular in mountain areas. The specificity of raw milk cheeses relies, among other things, on the preservation of microbial diversity *in situ*. To manage this microbial diversity through all the process from milk

production to ripened cheeses, it is important to have a better understanding of the microbial reservoirs and how the milk production practices affect the microbial counts and how technological process modifies the milk microbiota dynamics. The composition of the milk microbiota depends on the composition of the microbiota of sources directly in contact with the milk: the animal's teats and dairy equipment such as milking machine, milk line and tank. Concerning indirect sources, teat care, washing, and disinfection of the milking equipment are of primary importance (Vacheyrou *et al.*, 2011). As the surface of cow teats has been described as a source of microorganisms for milk, it can be hypothesized that practices that can modify the teat skin microbiota could be a lever to increase qualitatively and quantitatively the microbial diversity in milk. The objectives of this study, conducted in controlled conditions, were i) to evaluate if the post-milking treatment affects the teat and milk microbiota and ii) to compare these microbiota.

II – Materials and methods

This study was carried out at the experimental farm of INRA-UEMA Marcenat in an upland area of central France using 48 dairy cows, divided into three equivalent groups. At the end of each milking, throughout the whole lactation, the three groups received respectively: an iodinated product (I), commonly used in dairy farms with disinfecting action; a 85% glycerol product (G), selected for its lack of antiseptic activity and its power of regenerating the lipid layer; no product (O). Once a month the total surface of the four teats of each cow and the individual (harvested just after milking without refrigeration) milk were sampled. The individual milk samples (the total surface of the 4 teats) were pooled within groups of post-milking treatment. The microbial flora of each teat and milk pool (I, G, O) was counted on several culture media to enumerate: total mesophilic bacteria on Plate Count Agar medium (PCA); facultative heterofermentative *Lactobacilli* on agar medium FH; lactic acid bacteria on de Man, Rogosa and Sharpe (MRS) medium; *Enterococci* on Slanetz and Bartley agar medium (SB); yeasts and moulds on Oxytetracyclin Glucose Agar medium (OGA). The ripening flora was counted on Cheese Ripening Bacteria Medium (CRBM) and the presumed Gram negative flora (Gram -) on PCA medium with vancomycin and purple crystal added (PCAi). Two hundred fifty-three milk isolates and 319 teat isolates were picked up on culture media (mainly CRBM and PCA) inoculated with milk and teat pools at different sampling time. They were there identified by 16S rRNA amplifying-sequencing (Verdier-Metz *et al.*, 2012).Results of microbial counts at each sampling time were expressed as cfu/mL of teat juice or milk. The share of each microbial population in the total population was calculated as the ratio of the count of this population to the sum of the counted populations (CRBM + PCAi + SB + FH + OGA). The microbial counts were transformed to decimal logarithm to be analysed by univariate (post-milking treatment) analysis of variance using sampling times as replicates.

III – Results and discussion

The counts of lactic acid bacteria, Gram negative bacteria, yeasts and moulds were similar on teats, whatever was the post-milking treatment (Table 1). The lack of effect on these bacterial counts was also observed in milk. Teats treated with I and G post-milking product had a significantly lower count of ripening bacteria [less than 5.4 log(cfu/ml teat juice)] and consequently of total mesophilic bacteria [5.71 log(cfu/ml teat juice) than those without treatment [about 6 log (cfu/ml teat juice)]. On the contrary, such treatment had no effect on the count of these bacteria in milk. Thus, it can be hypothesised that the microbial count in milk is not directly affected by change of microbial count on teats. Even if the results are not expressed in the same units the levels of Gram- bacteria [about 3.25 log(cfu/ml milk)] and lactic acid bacteria [about 3.5 log(cfu/ml milk)] in milk were at higher levels than on teats whereas ripening bacteria were at lower levels. Interestingly the levels of yeasts, moulds, and heterofermentative *lactobacilli* in milk were at high-

er levels than on teats, whereas ripening bacteria were at lower levels. The microbial balance between teat and milk was quite different. On teat skin, ripening bacteria represented more than 96% of total population counted in different media, whereas they represented the same percent than lactic acid bacteria (31%) in milk. In milk the percentages of Gram-negative bacteria, yeasts and moulds were higher (14%) than on teat skin. This change in microbial balance between teats and milk suggests that the transfer of micro-organisms from teats to milk is hampered.

Table 1. Effect of post-milking treatment on teat (log cfu/ml teat juice) and milk (log cfu/ml milk) microbial counts over the lactation period

	Teat			Milk		
	I	G	O	I	G	O
Total mesophilic bacteria (PCA)	**5.71**^{b}	**5.71**^{b}	**6.17**^{a}	4.83	4.74	4.83
Ripening population (CRBM)	**5.30**^{c} (98.1)	**5.38**^{b} (96.5)	**6.08**^{a} (99.0)	3.51 (27.0)	3.44 (28.8)	3.50 (31.1)
Presumed Gram negative bacteria (PCAi)	3.22 (0.8)	3.21 (0.6)	3.16 (0.1)	3.36 (19.1)	3.23 (17.7)	3.17 (14.5)
Lactic acid bacteria (MRS)	3.33 (1.0)	3.84 (2.8)	4.00 (0.8)	3.55 (29.6)	3.45 (29.4)	3.50 (31.1)
Enterococci (SB)	1.31	1.53	1.26	1.13 (0.1)	0.81 (0.1)	0.81 (0.1)
Heterofermentative facultative *Lactobacilli* (FH)	1.52	1.82	1.79	3.06 (9.6)	2.95 (9.3)	2.96 (8.9)
Yeast (OGA)	1.79	1.59	2.00	3.02 (8.7)	2.92 (8.7)	2.95 (8.8)
Mould (OGA)	1.54	1.55	1.71	2.84 (5.8)	2.76 (6.0)	2.75 (5.5)

Results with different letters (a>b>c) are significantly different by the statistical Newman-Keuls test. Number in brackets is the percentage of this population in the (CRBM + PCAi + MRS + SB + FH + OGA) count.

Table 2. Microbial diversity of teat surface and milk

		Teat	Milk
Gram+ Catalase-	*Aerococcus, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Streptococcus*	x	x
	Desemzia, Pediococcus, Trichococcus	x	
Gram+ Catalase+ (ripening bacteria)	*Arthrobacter, Brachybacterium, Brevibacterium, Corynebacterium, Kocuria, Microbacterium, Micrococcus,Plantibacter, Rhodococcus,Staphylococcus*	x	x
	Deinococcus, Propioniciclava, Rothia		x
	Agrococcus, Bacillus, Cellulomonas, Citrococcus, Clavibacter, Curtobacterium, Dietzia, Exiguobacterium, Jeotgalicoccus, Macrococcus, Paenibacillus, Planococcus, Salinococcus	x	
Gram- Catalase-	*Erwiniia, Roseomonas*	x	
Gram- Catalase+	*Ochrobactrum, Pseudomonas, Raoultella, Stenotrophomonas*	x	x
	Agrobacterium, Chryseobacterium, Citrobacter, Comamonas, Delftia, Hafnia, Klebsellia, Phyllobacterium, Rahnella, Serratia, Yersinia		x
	Achromobacter, Aminobacter, Enterobacter, Escherichia, Pantoea	x	

Sequencing of the 16S rDNA of the picked-up colonies on culture media (mainly CRBM and PCA) according to their morphotype showed a wide diversity of microbial populations. Indeed, in the dominant population 43 microbial genera were identified on the teat skin, compared to 34 in the milk. Twenty genera in milk were in common with teat skin (Table 2). The teat skin microbiota differed mainly from that of milk by 13 microbial genera belonging to the ripening population (Gram+ Catalase+, CRBM). Fourteen genera found on teat skin have been detected in milk by other authors (Montel *et al*., 2014). Eight genera found on teat skin in our study had not previously been identified, neither in milk, nor on teat skin: *Agrococcus*, *Aminobacter*, *Cellulomonas, Citrococcus, Desemzia, Erwiniia, Planococcus, Roseomonas.* Two genera found in milk (*Propioniciclava* and *Phyllobacterium*) have never been previously described in milk.

These results confirmed that the teat skin can be considered as a reservoir of microbial biodiversity for raw milk. Nevertheless differences between teat and milk microbiota argue for other sources of milk inoculation (environment, milking machines). These results are in agreement with the composition of teat and milk microbiota in the literature (Montel *et al*., 2014).

IV – Conclusions

Our study showed that the post-milking treatment impacts only the ripening bacterial count (and consequently the total mesophilic bacteria count) on teats, but without modification of this count in milk. Teat and milk microbiota composition differed. The number of species belonging to the Gram+ Catalase+ group (ripening bacteria) was higher on teat skin than in milk. In milk percentages of lactic acid bacteria and ripening bacteria were similar to those of yeasts and heterofermentative lactobacilli. Our results confirmed that there are breaking points between animal and milk at a farm level. Further researchs are needed to identify precisely these breaking points and to better understand how microbial strains flow through the different ecosystems surrounding the animals and milk. The high throughput sequencing will facilitate such studies.

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