

Bacterial and botanical diversity of the pasture influence the raw milk cheese sensory properties

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Abstract

The interactions among plant and bacterial ecosystems of pastures, and livestock and their products, are intrinsic to quality schemes for protected designation of origin, but still lack in-depth comprehension. To study the transfer of bacteria from pastures to cheese, a highly biodiverse permanent grassland and an adjacent old temporary grassland were grazed by 2×3 balanced groups of 4 dairy cows each. A total of 18 Cantal-type cheeses were produced from raw milk (3 replicates per group) and ripened during 9 weeks. Bacterial community profiles (16S rDNA metabarcoding) differed significantly in the simulated herbage bites selected by dairy cows and to a lesser extent in raw milk and cheese depending on the pasture type. Sixty-seven bacterial sequence variants were shared between simulated bites, milk, and cheese core and rind. The most abundant sequence in cheese core and rind (assigned to *Lactococcus lactis*) was found also in simulated bites and in milk. Other sequences with above 8% abundance in cheese rind (assigned to *Brevibacterium aurantiacum* and *Brachybacterium* sp.) were also shared with simulated bites. The less firm texture of the cheeses from the highly biodiverse pasture could be attributable to their higher fat in dry matter content, whereas their stronger dry fruits odour and flavour could be partly explained by their specific bacterial community profile.

Keywords: biodiversity, 16S rDNA metabarcoding, raw milk cheese, flavour

Introduction

The botanical diversity of pastures has long been associated with the sensory characteristics of raw milk cheeses (Martin *et al.*, 2005). Nevertheless, evidence of the direct influence of the diversity of plant secondary compounds on the development of the cheese odour and flavour is often hypothesized, but was experimentally disproved (Tornambé *et al.*, 2008). The diversity of bacterial species in the phyllosphere in association with the botanical diversity of grasslands may also be a driver of the development of the raw milk cheese sensory quality (Frétin *et al.*, 2018). We hypothesize that the botanical diversity of the pastures shapes the microbiota along a continuum from the aboveground surface of plants to raw milk and eventually to raw milk cheese. A controlled replicated experiment with standardized cheesemaking procedures was carried out in order to characterize the bacterial communities from the herbage selected by dairy cows (i.e. simulated bites) to the ripened cheeses.

Materials and methods

The experiment was conducted at the INRAE Herbipôle experimental farm (<https://doi.org/10.15454/1.5572318050509348E12>). Twenty-four Holstein and Montbéliarde cows were randomly allocated to 6 groups of 4 cows, each balanced by breed, lactation number, milk yield, as well as milk fat and protein yields. Three groups were assigned to a highly biodiverse permanent grassland (HD; Shannon diversity index H' 312) and the 3 others to a low diversified adjacent old-temporary grassland plot (LD; H' 219). The cows were exclusively pasture-fed and had free access to NaCl and water. Three weeks of samplings followed 2

weeks of adaptation to the respective pasture. On one day per sampling week, samples of simulated bites of each group were collected by following the cows on pasture between morning and evening milking and collecting herbage samples with scissors by mimicking their selection for plant species and plant parts. Microbial contaminations were minimized by wearing gloves, and disinfecting scissors between different groups. The morning bulk milk of each group was collected once per sampling week, sampled for analysis and simultaneously processed to Cantal-type cheeses in an experimental cheesemaking facility (INRAE, UMR Fromage, Aurillac). After 9 weeks of ripening, the 18 cheeses were sampled for chemical and sensory analyses according to Manzocchi *et al.* (2021). Bacterial communities in samples of simulated bites, milk, cheese rind, and cheese core were characterized by 16S rDNA metabarcoding according to Fréтин *et al.* (2018). The amplicon sequence variants (ASVs) in each sample were identified and the H' indexes were calculated with the rANOMALY package in R (Theil and Rifa, 2021). The data were analysed with a linear mixed model (mixed procedure in SAS, version 9.4) including pasture type as fixed effect and group (all data), panellist, and session (only for sensory data) as random effects.

Results and discussion

Cheeses derived from the botanically highly diverse pasture were tendentially more yellow and less firm, and had a higher fat in dry-matter content, as well as more intense 'dry fruits' odour and flavour (Table 1). No differences were observed in the spreadability index (*cis*-9 C18:1-to-C16:0 ratio) of the cheeses between the pasture types. A higher total number of bacterial ASVs and a higher bacterial H' index were found in the bites selected on the botanically highly diverse pasture than in those selected on the less diverse pasture (Table 2). The total number of ASVs and the H' index of milk, cheese core, and cheese rind did not differ significantly between pasture types. Simulated bites, milk, as well as cheese core and rind derived from the two pasture types had 67 common ASVs, 15 of which were found in all analysed compartments regardless of the botanical diversity of the pasture (Figure 1). Among the latter, an ASV assigned to *Lactococcus lactis* with a very high relative abundance in the cheese core (93.9%) and cheese rind (49.4%) was found with lower abundances also in milk (5.9%) and in simulated bites (0.2%). Two ASVs assigned to *Brevibacterium aurantiacum* and *Brachybacterium sp.*, both notably involved in cheese ripening, were very abundant in the cheese rind (17.3%) and were identified with lower abundances also in all other compartments, which might indicate a possible transfer of these bacteria from the pastures to raw milk and cheese.

Conclusions

In conclusion, the less firm texture of the cheeses from the highly biodiverse pasture may be attributable to their higher fat in dry matter content, their more intense 'dry fruits' odour and flavour could be partly

Table 1. Effect of the botanical diversity of the pasture on the cheese composition, proteolysis, colour of the curd, fatty acid (FA) composition, and sensory properties.

	Botanical diversity		SEM	P-value
	High (HD)	Low (LD)		
Fat in dry matter, g 100 g ⁻¹	51.4	47.5	0.44	<0.001
Water-soluble N / total N, g 100 g ⁻¹	34.0	36.4	4.13	0.705
Phosphotungstic acid soluble N / water-soluble N, g 100 g ⁻¹	30.3	32.1	0.72	0.074
Phosphotungstic acid soluble N / total N, g 100 g ⁻¹	10.3	11.7	1.52	0.543
Brightness of the curd, L* index	74.5	70.9	1.02	0.068
Yellowness of the curd, b* index	24.1	21.4	0.72	0.053
Spreadability index (<i>cis</i> -9 C18:1-to-C16:0 ratio)	0.95	0.92	0.05	0.666
Firmness, sensory score 0-10	5.7	6.4	0.27	0.092
Global flavour, sensory score 0-10	6.0	5.6	0.13	0.128
Dry fruits odour, sensory score 0-10	3.2	2.6	0.13	0.038
Dry fruits flavour, sensory score 0-10	3.1	2.5	0.11	0.004

Table 2. Effect of the botanical diversity of the pasture on the number of ASVs and the Shannon diversity index (H') in simulated bites, milk, cheese core, and cheese rind.

	Number of ASVs				Shannon diversity index (H')			
	High (HD)	Low (LD)	SEM	<i>P</i> -value	High (HD)	Low (LD)	SEM	<i>P</i> -value
Simulated bites	295.8	242.3	14.18	0.016	4.21	3.70	0.102	0.002
Milk	144.6	113.6	15.41	0.163	2.35	2.74	0.348	0.462
Cheese core	44.1	35.6	3.87	0.141	0.40	0.34	0.042	0.325
Cheese rind	44.6	37.5	2.66	0.083	1.57	1.59	0.060	0.816

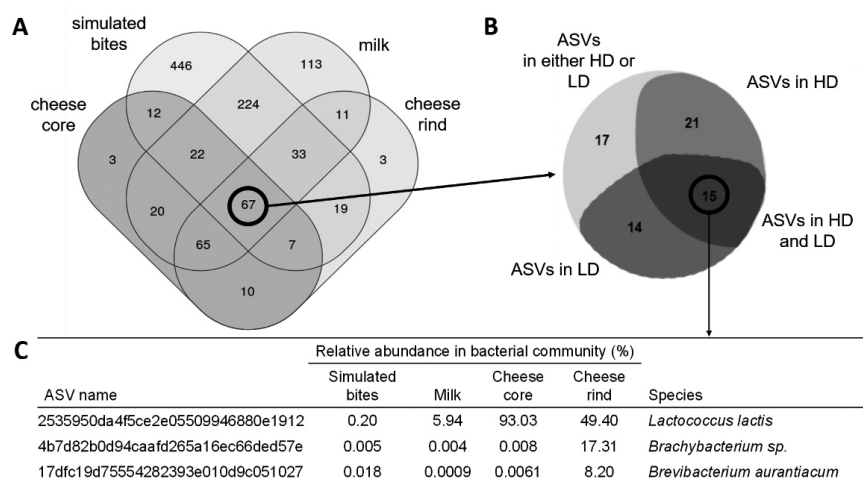


Figure 1. Number of ASVs shared between bacterial compartments derived from the two pasture types (A), distribution of common ASVs according to the pasture type (B), and relative abundance in the bacterial communities of the main ASVs that were common to the two pasture types (C).

explained by their specific bacterial community profile. Eventually, the botanical diversity of pastures may contribute to the shaping of the bacterial communities of milk and cheese through the transfer of bacteria from the grassland's surface to raw milk. Other microbial communities (i.e. fungi) and ecosystems, such as the soil, the rumen, as well as the teat-skin surface, will be investigated to unravel the potential pathways of microbial transfer.

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