Bacterial and farm management risk factors in raw milk cheese production ?

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INTRODUCTION

- Numerous factors can affect the sanitary quality of raw milk cheeses : cow's diet, mountain pasture, animal microbiota (e.g. udder surface, teat canal, faeces), housing conditions, cheese production environment (e.g. water, contact materials, ripening racks, milking machine and milking machine cleanliness, air in the barn), season.
- Preventive and corrective measures are recommended, such as pre-harvest interventions (milking hygiene practices or the use of probiotics).
- Risk assessment suffers from gaps related to the circulation of bacterial strains at farm level and during cheese production, and to the identification of optimal hygiene and manufacturing practices.
- Study objectives: 1/ evaluate potential risk or protective factors from both practices and the bacterial ecosystem in French artisanal raw milk cheese production and 2/ identify critical control points, using a retrospective control case study.

METHODS

10 case farmhouses production

10 control farmhouses production

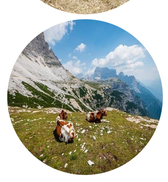
With the highest number of exceedances of the criteria set by Regulation (EC) No 2073/2005, including the detection *of L. monocytogenes*, Shiga toxin-producing *E. coli*, and *Salmonella* spp. (2017 to 2022)

With good food safety criteria (2017 to 2022)

Two 4-weeks sampling campaigns C1 and C2

C1 - 2022 Winter

789 environmental and product samples before and after starter addition



C2 - 2023 Summer

40 completed questionnaires on farming and cheesemaking practices

16s rRNA metabarcoding

Selection of variables by univariate analysis (nVC = number of variables per campaign included in Multiblock Partial Least Squares analysis (MB-PLS))

MB-PLS conducted in R (ade4 package) to explain the block of Y (control or case farms) (Bougeard and Dray, 2018) by a large number of explanatory variables, divided into 4 blocks:

Block_1 (B1)	Block_2 (B2)	Block_3 (B3)	Block_4 (B4)
nVC1=96 nVC2=56	nVC1=57 nVC2=147	nVC1=5 nVC2=22	nVC1=3, nVC2=2
Environmental and product samples collected before starter addition	Same variables as in B1 collected after starter addition	Farming practices	Cheesemaking practices

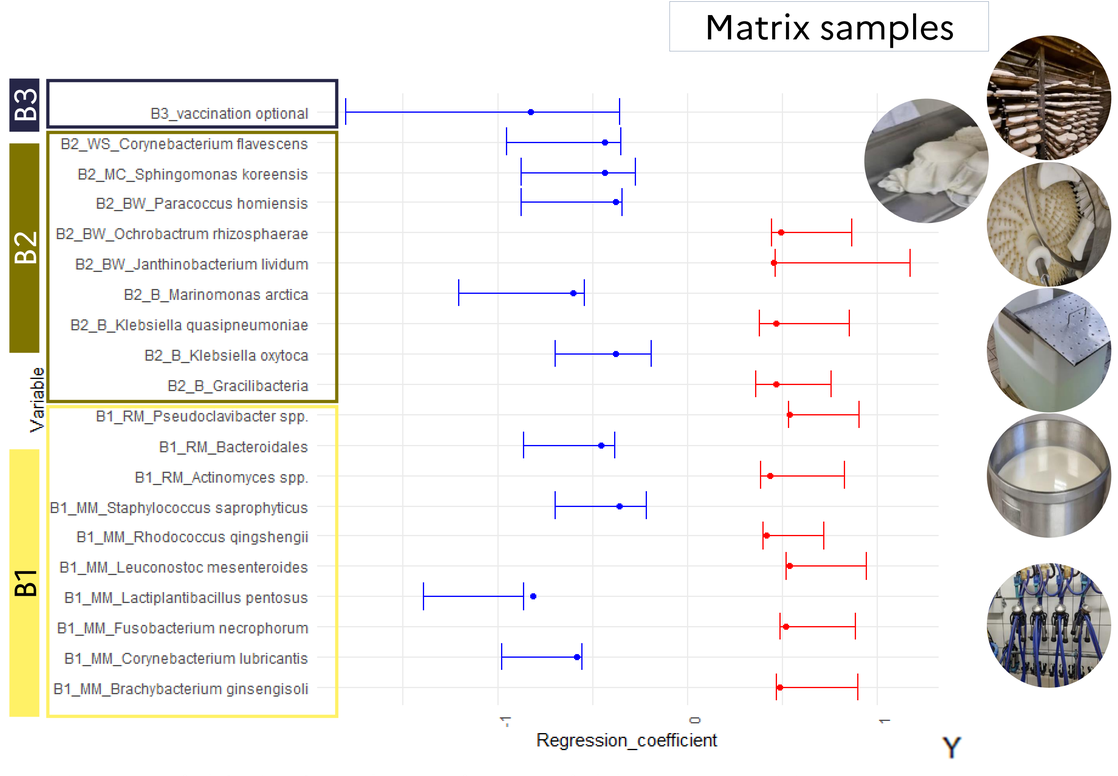
Y: case, control

pH
Temperature

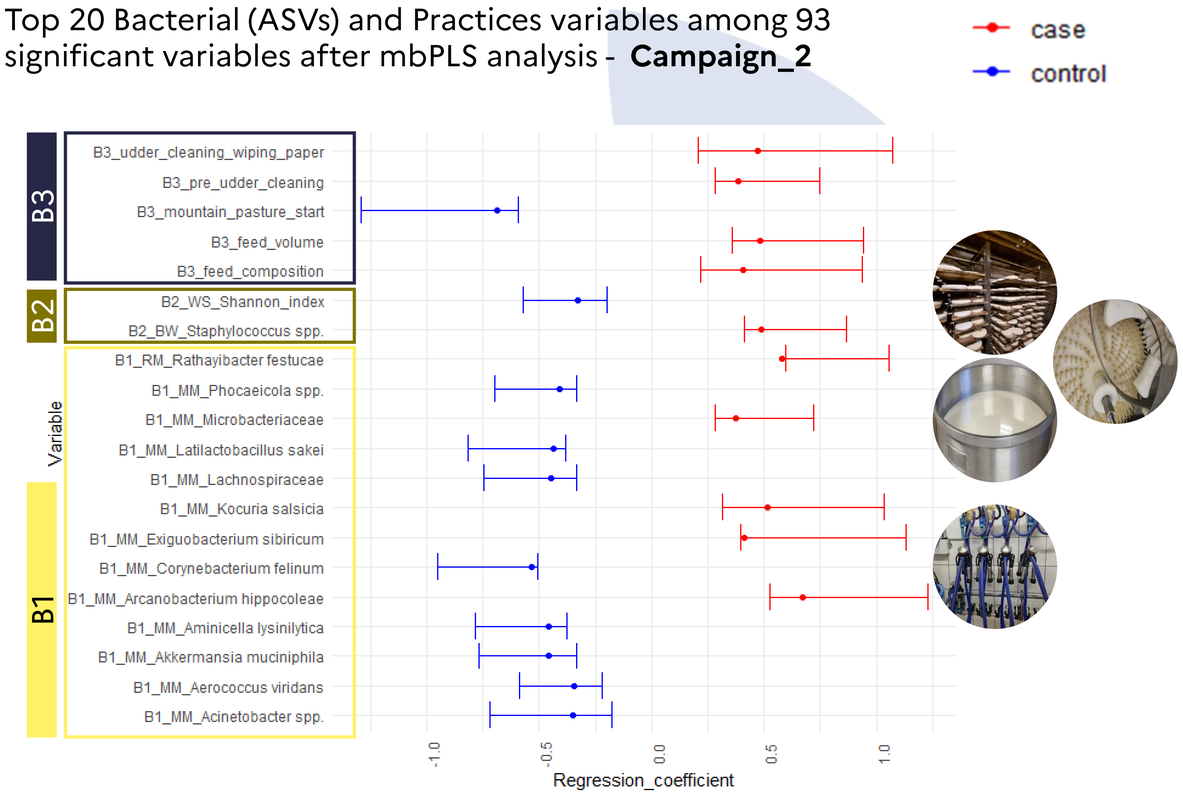
Bougeard, S., and Dray, S. (2018). Supervised Multiblock Analysis in R with the ade4 Package. *Journal of Statistical Software*, *86*, 1–17. https://doi.org/10.18637/jss.v086.i01

RESULTS

Top 20 Bacterial (Amplicon Sequence Variants, ASVs) and Practices variables among 52 significant variables after mbPLS analysis - **Campaign_1**



Top 20 Bacterial (ASVs) and Practices variables among 93 significant variables after mbPLS analysis - **Campaign_2**



95 % confidence interval (CI) of the mean difference calculated by performing bootstrap. WS wooden shelves, MC moulding cloth, BW Brush washing cheese, B brine, RM raw milk, MM milking machine

✓ Microbiological criteria set by regulation not exceeded during sampling period

CONCLUSION

- Despite the limited number of farms studied in this specific artisanal production, this work identified matrix samples that could be considered as critical sampling points: milking machine, raw milk, brush washing cheese and wooden shelves, both in winter and in summer.
- These matrices harbored bacterial taxa (ASVs) associated with either case or control farms.
- Significant ASVs mostly identified in environmental samples and raw milk.
- Significant ASVs changed with winter and summer practices, with the exception of *Corynebacterium* spp.
- In winter, voluntary additional vaccination of dairy cows may serve as a protective measure against pathogenic bacterial contamination.
- In summer: (1) higher alpha diversity (Shannon index) on wooden shelves may act as a protective factor, (2) farm practices (use of paper for udder cleaning or increased feed mix) may be risk factors for pathogenic bacterial contamination, whereas transhumance to mountain pastures is a protective factor.
- This study highlights (1) the importance of understanding the dynamics and impact of bacterial communities in production environments to further improve food safety and quality, and (2) the value of multi-block analysis in managing complex datasets, suggesting that 16S rRNA markers from metabarcoding can help identify critical control points and risk factors.