

THE CHANGING CHEESE MICROBIOME

Cheese microbiome dynamics during cheese manufacturing and ripening.

Modern Danish industrial cheese production faces the challenge of maintaining constant premium cheese quality, even when production is moved to different locations, or the milk base is changed to drive more cost-efficient cheese production.

Understanding the cheese microbiome

Many of the flavour and texture characteristics that cheese makers aim for result from an interplay between the specific properties of the milk used as a base, with the different microbes that are present within the cheese as it transforms from the initial milk into its final product. Although the general relevance of these microbes (its so-called microbiome) has been long known and appreciated, today we have the tools to profile them in detail. In this way we can begin to understand not only exactly which microbes are present and what relevant genetic properties they might have, but also how and when they enter the manufacturing process, how they are shaped by different sources and pretreatments of milk, and how their relative abundances change throughout the manufacturing process itself. Given that ultimately understanding the cheese microbiome is key to optimising and standardising the quality in the Danish dairy industry, we applied the latest DNA sequencing tools to explore



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both how different sources and pretreatments of milk, as well as variation in ripening temperature shape this community in Herrgaard cheese at two different manufacturing locations, Arla amba's Skejby pilot plant and the commercial Kalmar facility.

The milk base matters

Thanks to having access to samples spanning raw milk to final cheese, our first major observation was that both the milk source itself, and its pre-treatment really does affect the microbes! For example, while regardless of origin, all raw milk samples contained some microbes – including the known spoilage bacteria *Janthinobacterium*, subsequent microfiltration considerably reduced the overall microbial level (Figure). In contrast, we also noted that, while fortification is a useful tool for modifying the milk properties and boost yield, it does not come without risk. For example, in at least one case we found it led to contamination with *Streptococcus*. Unfortunately, potentially problematic microbes were not only associated with the raw milk, but also the pre-treatment method applied. We also were able to obtain early detection of several samples with low levels of *Clostridium tyrobutyricum* and follow their contribution to the cheese microbiome throughout the cheese manufacturing process. Nevertheless, microfiltration was able to make *Clostridium* practically undetectable (see

Abstract:

Without microbes, there is no cheese. While certain microbes are deliberately added to the milk base early in the manufacturing process, others also contribute to cheese-making process with both positive and negative consequences. But which microbes are these and how do their abundances change as the milk becomes cheese? And where do they come from and how do they enter the manufacturing process? By applying state-of-the-art DNA profiling techniques to study these questions in Herrgaardsost produced at two different facilities, we provide new insights into these questions.



figure). This highlights a potential future where the use of DNA-based tools can be used as a foundation for the cheese makers to guide the use of the raw milk, or the cheese ripening so that late blowing is not developed, and food waste is avoided in the dairy industry. Similarly, we were able to identify several phages (viruses that target and kill very specific bacteria) that entered the process with the milk at both locations. Although the relevance of the phages is not clear at this time, given the increasing interest in phages both in the context of spoilage (because they kill beneficial microbes) but also as a tool for eliminating problematic microbes, their future study may be rewarding.

Differentiation by ripening temperature

Naturally our analyses were not only limited to potentially problematic microbes, but also provided insights into those that are critical to the final cheese quality. For example, while our analyses revealed that, overall, the microbial community contained fewer than 10 different species, unsurprisingly there was an immediate effect of the addition of the starter culture (in this case, a blend of *L. lactis*, *L. cremoris*, *L. lactis* biovar *diacetylactis* and *Leuconostoc*) on the overall microbial community. In addition to rapidly taking over the community, their own relative abundances changed throughout the process, with the initially rarer *L. lactis* ultimately dominating the whole community (-see figure). This relationship was remarkably consistent across our test samples, regardless of milk base, geographic location, ripening temperature and even location in the cheese (rind vs core) (Figure), highlighting the relevance of maintaining a high control over the starter culture should consistency be an end goal. Nevertheless, despite this apparent consistency when ripening temperatures were varied from 13°C and 20°C, biochemical data that we also generated did reveal clear differences related to the formation of aroma compounds and the overall proteolytic level of the cheeses, both important elements for the flavor and quality of Herraardstost. To explore this further we plan as a next step to explore the functionality of the cheese microbiome using RNA sequencing – something we can now do having established a robust laboratory and computational pipeline to process such data from cheese. In doing so we will be able to highlight how the expression of the genes in the microbes are up or down regulated in relation to parameters such as the ripening temperatures. ●

Project facts:

Title: From cheese microbiome to robust cheese making processes (MetaCheese)

Objective: To use a multi-omics approach to map the microbial composition during cheese making and ripening to understand the functional role in relation to cheese quality and flavor.

Project manager: Professor Tom Gilbert, Research Director, Section for Evolutionary Genomics, GLOBE Institute, University of Copenhagen (UCPH).

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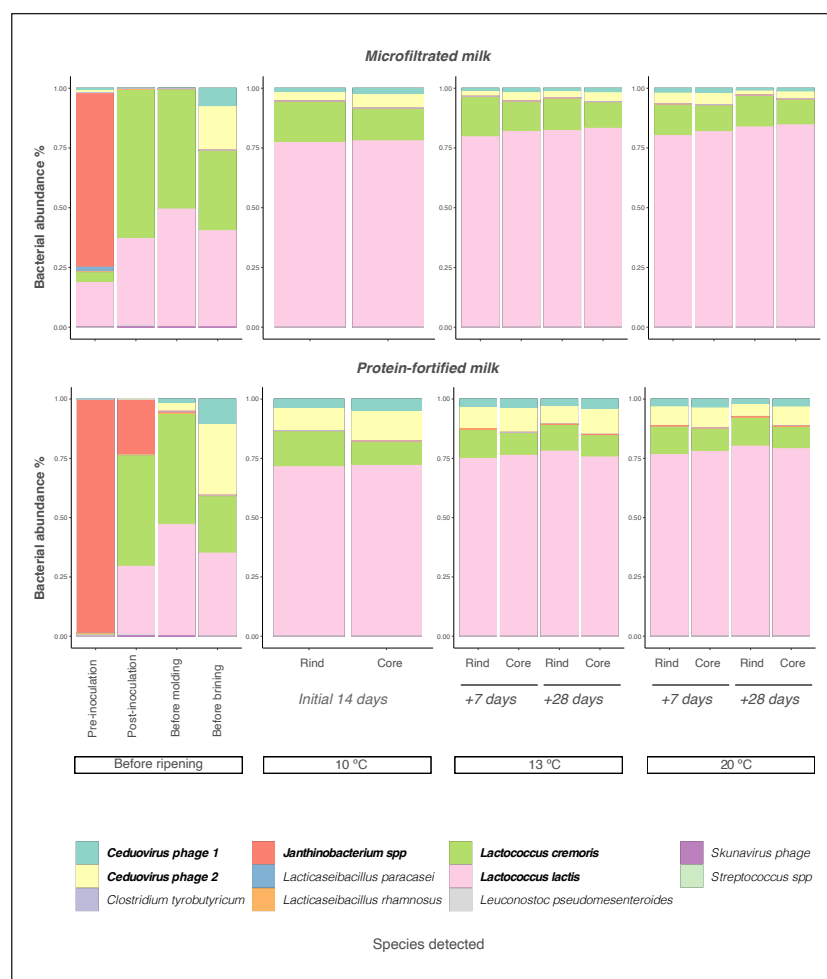


Figure legend: Using the latest DNA sequencing techniques allows detailed profiling of not only which microbes are present at the different steps of the cheese making process, but how their relative abundance changes through time. This in turn can provide critical insights into both where they come from, and how they shape the cheese making process.