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Tom Gilbert:

Fra oste-mikrobiom til robuste osteprocesser (MetaCheese)

From Cheese microbiome to robust cheese making processes

Mejeribrugets
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Final report

for collaborative projects funded via the Danish Dairy Research Foundation (DDRF)

1. Title of the project

In Danish: Fra oste-mikrobiom til robuste osteprocesser (MetaCheese)

In English: From Cheese microbiome to robust cheese making processes

2. Project manager

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***Postdoc who joined the project near the end to assist process extra data.

4. Sources of funding

Milk Levy Fund

Chr. Hansen A/S (cofinancing)

Arla Foods amba (cofinancing)

University of Copenhagen (extra cofinancing with relation to salary of Juan Antonio Rodríguez Pérez)

5. Project period

Project period with DDRF funding: 01 Jan 2019 – 31 Dec 2020

6. Project summary

In Danish:

Formål: Selvom det er afgørende for den industrielle produktion af ost at opretholde konsistensen af slutproduktet, er det velkendt, at små ændringer i produktionen ofte kan have store konsekvenser for den resulterende ost. Faktorer, der kan påvirke processen på denne måde, inkluderer blandt andet mælken, der bruges til at fremstille osten, den anvendte starterkultur, forskelle i udstyr på mejerierne, og endda mælkens oprindelse, og hvordan denne mælk evt. er forbehandlet eller ej. For bedre at forstå dette samspil mellem faktorer gennemførte vi en række eksperimenter, på to geografiske lokaliteter (Kalmar, Sverige og Skejby, Danmark), med det formål at undersøge, hvordan den mikrobielle sammensætning af Herrgårdsost ændrer sig både gennem selve produktionsprocessen, men også i forhold til den geografiske placering og variation i mælkekilden.
Resultater: Helt konkret indsamlede vi et stort antal prøver, som spænder over op til 12 punkter i ostefremstillingsprocessen, over i alt fem forsøg udført på de to forskellige geografiske lokaliteter. Punkterne for prøveudtagningen strakte sig fra den første mælk før tilsætning af starterkulturen, til modning af osten ved to forskellige temperaturprofiler. Fra alle disse prøver genererede vi metabolomiske (biokemiske), metagenomiske (mikrobielle DNA) og metatranskriptomiske (mikrobielle RNA) data, som derefter blev testet i en række analyser. Resultaterne har foreløbigt givet indsigt i (i) det oprindelige mikrobielle samfund i mælken før modtagelse på faciliteterne og efter den indledende behandling, herunder tilstedeværelsen af potentielt skadelige bakteriofager, (ii) effekten af mikrofiltrering ved mælkens ankomst, (iii) hvordan det mikrobielle samfund formes under ostefremstillingsprocessen, især med hensyn til den relative overflod af starterkultur *L. lactis* vs. *L. cremoris*, (iv) at der er bemærkelsesværdig sammenhæng mellem skorpe- og kernesamfundene af ostene, og (v) at der generelt er betydelig stabilitet i de mikrobielle samfund under hele modningsprocessen.
Konklusioner: Vores analyser giver indtil videre vigtig indsigt i kilderne til mulige problematiske mikroorganismer i ostefremstillingsprocessen, samt viser en bemærkelsesværdig stabilitet i det mikrobielle samfund i både tid og rum. Desuden var datagenereringen i sidste ende mere vellykket end forventet, på grund af tilfældige forbedringer i laboratoriemetoderne. Derfor forventer vi, at yderligere analyser vil blive udført i løbet af det kommende år, med særligt fokus på at bruge de metatranskriptomiske data til yderligere at udforske funktionen af de identificerede mikrober i forhold til de metaboliske og øvrige producerede sensoriske profiler.

In English:

Aim: Although maintaining consistency of the product is critical to the industrial production of cheese; it is well known that often small changes in the production can have major consequences on the resulting cheese. Among other things, factors that can affect the process in this way include the actual dairy used to make the cheese, the starter culture used, differences in the equipment in the facilities, and even the sources of the milk and how this milk might be pre-treated or not. To better understand this relationship, we undertook a series of experiments, at two geographic locations (Kalmar, Sweden and Skejby, Denmark) aimed at exploring how the microbial composition of Herrgårdsost changes both throughout the production process itself, but also in relation to geographic location and milk source variation.
Results: Specifically, we obtained a large number of samples spanning up to 12 points in the cheese making process, over at total of 5 trials performed at the 2 locations. Sampling points spanned from initial milk prior to the addition of starter culture, through to maturation of the cheese at two different temperature profiles. From all these samples, we generated metabolomic (biochemical), metagenomic (microbial DNA) and metatranscriptomic (microbial RNA) data, which were then subjected to a suite of analyses. The findings so far have provided insights into (i) the native microbial community of the milk prior to receipt at the facilities and post initial treatment, including the presence of possibly detrimental phages, (ii) the effect of microfiltration upon milk receipt, (iii) how the microbial community is shaped during the cheese making process, in particular with regards to the relative abundance of the starter culture *L. lactis* vs *L. cremoris* microbes, (iv) that there is remarkable consistency between the rind and core communities of the cheeses, and (v) that in general there is considerable stability in the communities throughout the ripening process.
Conclusions: While our analyses so far show important insights into the sources of possibly problematic microbes in the cheese making process, as well as a remarkably stability in the microbial community both in space and time, ultimately the data generation was much more successful than anticipated, due to fortuitous developments in the laboratory methods. Hence, we anticipate additional analyses will be performed over the upcoming year, with a particular focus on using the metatranscriptomic data to explore further the function of the microbes identified, in relation to the metabolic and other sensory profiles produced.

7. Project aim

Dansk

Industriel ostefremstilling er afhængig af et komplekst samfund af mikroorganismer, oste-mikrobiomet, der dels stammer fra de tilsatte starterkulturer, mens den øvrige del stammer fra mælken samt fra mejeriets "husflora", der findes i bl.a. saltlagen og i produktionsapparatet. Når den mikrobiologiske sammensætning i ostene forandres, har det betydning for kvalitet og smagsdannelse i osteprocessen. Nøglebegivenheder i processen er derfor der, hvor disse forandringer foregår.

Vi foreslår at bruge en multi-omics tilgang ved at anvende metabolomics (biokemisk profilering); metagenomics (DNA baseret karakterisering af mikrobiomet); Metatranscriptomics (RNA baseret karakterisering af microbiomets aktivitet) til at forstå betydningen af de individuelle komponenter i ostemikrobiomets betydning for smagsdannelse og andre kvalitetsmål, når ost fremstilles i industriel skala. Mere specifikt vil vi undersøge betydningen af:

- Mejeriet: Brug af samme starterkultur i forskellige mejerier og i forskellige ostekar i samme mejeri.
- Mælkens mikroorganismer: Brug af samme starterkultur med filtreret vs. ufiltreret mælk, med forskellige mælkeindvejninger samt ved mælk fra forskellige årstider.
- Saltlagen: Samme starterkultur i høj saltkoncentration og ved lav saltkoncentration.
- Brug af modningskulturer: Samme syrningskultur med/uden brug af modningskultur.

English

Industrial cheese making is dependent on a complex community of microorganisms, the cheese microbiome, that partly derives from the added starter culture, but also those originating from the milk as well as the dairy's 'house-flora', that is found in, among others, the salt bath and production equipment. When the microbial composition of the cheese changes, it has a consequence for the quality and taste development in the cheese making process. Key knowledge in this process is therefore, where these changes happen.

We propose to use a multi-omics approach consisting of metabolomics (biochemical profiling), metagenomics (DNA based characterization of the microbiome), metatranscriptomics (RNA based characterizing of the microbiome's activity) to understand the relevance of the individual components in the cheese microbiome with regards to the taste development and other quality metrics when cheese is made on the industrial scale. More specifically we will explore the meaning of:

- The dairy/cheese vat: Use of the same starter culture in different dairies and in different cheese vats in the same dairy.
- The milks microorganisms: Use of the same starter culture with filtered vs unfiltered milk, with different milk providers and with milk from different seasons.
- Salt bath: The same starter culture in high salt vs low salt concentration.
- Use of ripening cultures: The same acidification culture with and without use of ripening culture.

8. Background for the project

The MetaCheese project was created based on the prior "FoodTranscriptomics" consortium, which was a 4-year Innovation Fund-supported project run by Chr Hansen A/S, and involving the MetaCheese team from University of Copenhagen, as well as other partners from Department of Plant and Environmental Science, University of Copenhagen; DTU Health Tech (former DTU-CBS), EMBL Heidelberg and Free University Amsterdam. This prior project had developed tools to understand and modify the cheese microbiome in a desired direction, thereby reducing the percentage of defective cheeses.

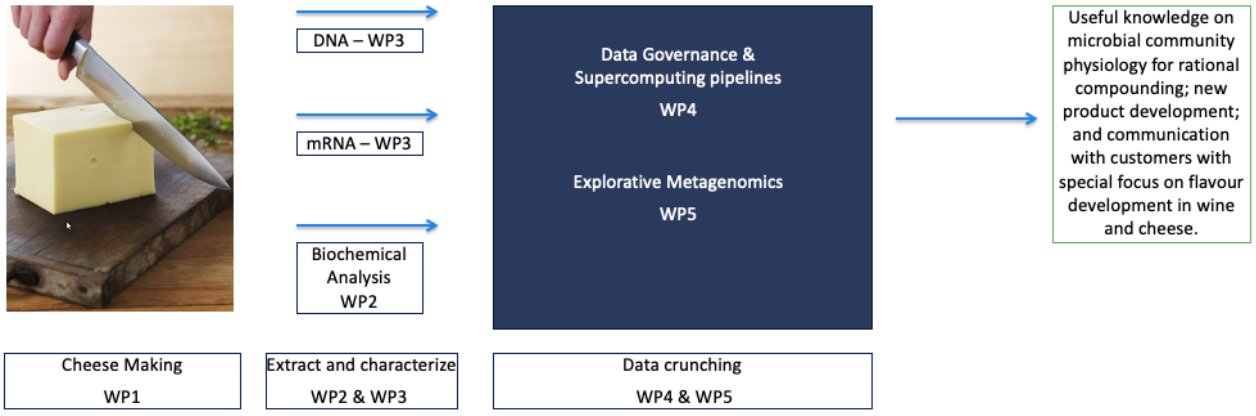
In light of the findings of the Foodtranscriptomics project, our consortium represented in the MetaCheese project proposed that it should be possible to take the tools developed and apply them to the well-known problem of reproducibility in industrial cheese making – namely that often certain changes in the cheese making process can have major consequences on the quality of the cheese produced. Typically, such changes can involve the major, e.g., change of actual cheesemaking location, but also factors such as change of milk supplier, season of milk production, changes in the way cheesemaking locations are cleaned, and of course changes in the starter cultures used in the processing.

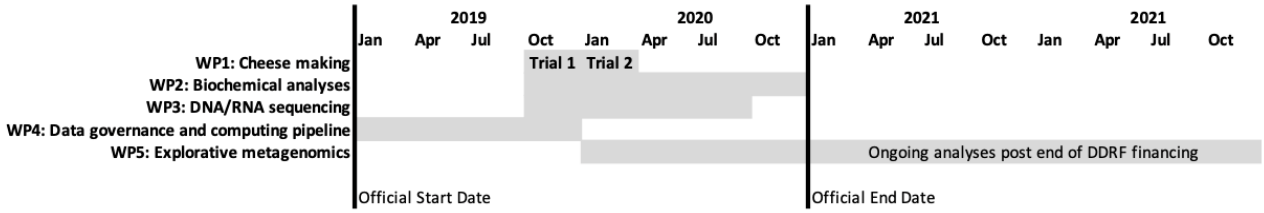
Given this, and thanks to the ability to work at both Arla Foods amba's pilot plant in Jutland as well as their industrial plant in Kalmar, we proposed to create a research project that would explore the effect of changing several parameters on the subsequent microbiome of the cheese. By using a wide range of techniques, including metabolomics (to profile the cheese quality), microbiomics (to profile the microbes in the cheese making process), and metatranscriptomics (to profile the activity of the microbes), we aimed to decipher what was going on.

Ultimately, we were able to generate an enormous amount of data – in fact much more than originally planned. The consequences of this have allowed us to make numerous observations so far, and indeed we are not yet finished analyzing the data to the full, hence the decision to involve a further staff member post the official completion of the original project.

9. Sub-activities in the entire project period

Metacheese was structured around 5 interconnecting work packages (WPs), as shown in the figure below. Also indicated is the eventual timeline of the activities, indicating how we elected to extend the activities through financing provided by the principal investigator (Gilbert) to allow extra analyses on the data. These WPs and the eventual timeline are shown below.





10. Deviations

The principal deviation was that the data generation was much more successful than anticipated, leading to a very large amount of information. This could not all be processed during the time of the original DDRF funded postdoc (Siren) as such as second postdoc, funded by PI Gilbert using discretionary funds, has joined the team, and continues to analyze the data to maximize the gain. It is anticipated this will lead the creation of several peer review publications in the next year or two, as well as provide important feedback to the industry. Beyond the above, there were small deviations in sampling strategy in light of the possibilities while running the trials. These are detailed further in section 11.

11. Project results

Introduction and sampling deviations:

In our original application we proposed to specifically target the following questions, all relevant to the quality of cheese produced in the industrial manner.

1) What is the effect of the dairy and cheese vat? This was to be tested by using a single starter culture in different cheese vats and dairies.
2) What is the effect of the microorganisms present in the milk prior to the process? This was to be tested using a single culture on different sources of milk including (a) with filtered vs unfiltered milk, and (b) different milk providers, and (c) milk from different seasons
3) What is the effect of the salt bath? This was to be tested by applying the same starter culture in high salt vs low salt concentration baths.
4) What is the effect of ripening cultures? This was to be tested considering the addition or exclusion of ripening cultures.

However, as mentioned in section 10, after initiation of the project, and considering various constraints with regards to which cheese making facilities were available, which suppliers were available, and in light of newly identified needs by the industrial partner, we ultimately elected to modify our aims as follows – changes indicated in red.

1) What is the effect of the dairy and cheese vat? No change. This was to be tested by using a single starter culture in different cheese vats (2-3 per dairy) and dairies (2 locations, Skejby and Kalmar).
2) What is the effect of the microorganisms present in the milk prior to the process? Minor change to expand aims. This was to be tested using a single culture on different sources of milk including (a) with filtered vs unfiltered milk, and (b) different milk providers, (c) milk from different seasons and (d), protein fortified vs unfortified milk.
3) What is the effect of the salt bath? This was to be tested by applying the same starter culture in high salt vs low salt concentration baths. Major change – as it was not practical at the facilities to use multiple salt baths, we elected to reduce focus on this and replace it with studying the variation between core and rind of the cheese when subjected to a single bath.
4) What is the effect of ripening cultures? This was to be tested in light of the addition or exclusion of ripening cultures. Changed – instead of studying effect of ripening cultures we elected to instead explore effect of ripening temperature, thus all our cheeses made ultimately were stored in different temperatures, as outlines in WP1.

Considering the above, we elected to change our sampling strategy in WP1, details of which follow.

WP1 Cheese making:

In early 2019 we performed our first cheese making experiment at Arla Foods' Research and Innovation Facility in Skejby, Denmark, under the lead of partner Arla Foods, with participation from Chr. Hansen A/S and University of Copenhagen. The details were as follows, with a summary shown in the below figures 1 & 2:

Target cheese: Herrgårdsost

3 milk bases: Micro filtrated (MF), Pasteurized (LP), Protein fortified (P4.2)

1 starter culture: ES-Flora C1060

Structure: 2 production days with 3 vats each day, 9 cheeses from each vat

2 ripening temperature profiles tested: 10 days at 10°C, 35 days at 13°C (Temperature 1) and 10 days at 10°C, 35 days at 20°C (Temperature 2).

Sampling at 10 timepoints: 1. Cultures, 2. Milk – receipt, 3. Post-pasteurization, 4. Pre-inoculation, 5. Post-innoculation, 6. Start heating and stirring (W+G), 7. Process water, 8. Before molding (W + G), 9. Before brining + sample of brine, 10. After 14 days at 10°C, 11. After 7 days at 13°C/20°C, 12. After 35 days at 13°C/20°C.

In January 2020 a cheese making experiment at industrial scale was conducted at Arla Foods facilities in Kalmar, Sweden, under the lead of the partner from Arla Foods, with participation from Chr. Hansen A/S and Copenhagen University. The details were as follows, with a summary shown in the below figure:

Target cheese: Herrgårdsost

Vat size: 21,000-liter tanks

Milk base: Milk from Øland (MF), Milk from Kalmar (MF)

Starter culture: ES-Flora C1060

Structure: 1 production day with 4 vats. 2 vats of each milk base.

2 ripening temperature profiles tested: 10 days at 10°C, 35 days at 13°C (Temperature 1) and 10 days at 10°C, 35 days at 20°C (Temperature 2).

Samples at 12 timepoints: 1. Cultures, 2. Milk – receipt, 4. Pre-inoculation, 5. Post-inoculation, 6. Start heating and stirring (W+G), 7. Process water, 8. Before molding (W + G), 9. Before brining and sample of brine, 10. After 14 days at 10°C, 11. After 7 days at 13°C/20°C, 12. After 35 days at 13°C/20°C.

Figure 1: Summary of the dataset structure including (right) ripening profile

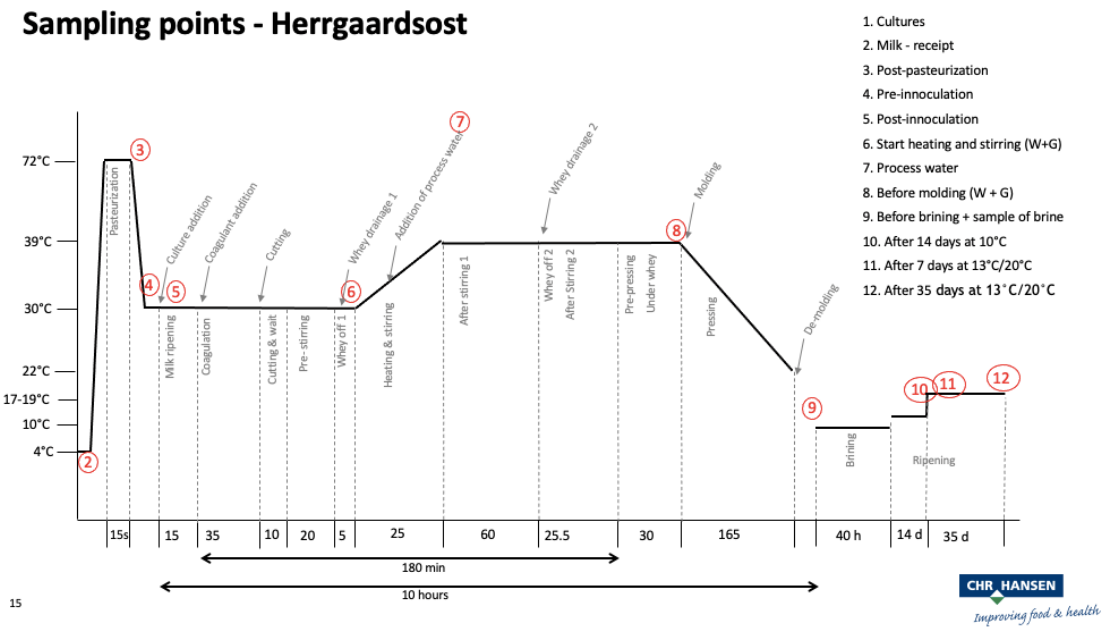


Figure 2: Details of the sampling profile.

Samples taken were provided to both WP2 and WP3, as detailed below.

WP2 Biochemical Analysis:

The strategy for the biochemical analysis was to document the effect of microbial activity on the conversion of mainly carbohydrates and proteins. A complex analytical package was applied to document specifically glycolytic and proteolytic activities as well as the interactions that naturally occur between the components formed. This included water-soluble and volatile components. Analyses were performed at Arla Foods amba as well as Chr. Hansen A/S, and in this way, we utilized the competences from the different project partners. All biochemical data has been collected in 1 file and shared with WP5 for further data analysis. Some examples on biochemical differences observed:

The analysis of volatile compounds showed an unset (before brining) of the production of the volatile compounds diacetyl and acetoin, which are important for the buttery aroma that characterizes a Herrgaardsost. For cheeses ripened at 13°C diacetyl and acetoin were identified through the whole ripening period of 7 weeks, whereas cheeses ripened at 20°C contained significant lower levels or even depletion of diacetyl and acetoin. The formation of these compounds is linked to the activity of the starter culture.

The primary proteolysis of the cheeses was primarily influenced by ripening time and to a smaller extend the ripening temperature. In addition, the level of intact casein was found to be comparable between cheese produced with different milk bases. On the other hand, the level of free amino acids (the secondary proteolysis) was clearly influenced by both ripening time and temperature. Overall, the high ripening temperature seems to boost the peptidase activity and to a lesser extend the protease activity.

WP3 DNA/RNA sequencing:

Research Assistant Luisa Nielsen at University of Copenhagen extracted DNA and RNA from the samples collected, that were converted to Illumina sequencing libraries and sequenced at Novogene UK. This required development of an optimized pipeline to maximize the recovery of nucleic acids from these challenging samples. The data was returned to CPH in August 2020, and initially analyzed by Postdoc Kimmo Siren, then following the close of his contact, Postdoc Juan Rodriguez (WP5).

WP4 Data Governance and Supercomputing pipelines

We developed a reproducible computational workflow to study the population genetics in cheesemaking covering from the raw data curation to the following data analysis. The workflow uses life science supercomputing and merges both reference-based and reference-free methods for genome generation allowing functional profiling of both model and non-model prokaryotes. This is done by combining the *de novo* assemblies from metagenomic species and isolated cultures, with a follow-up a set of strategies of aligning sequencing reads to these genomes. The genomes can be taxonomically and functionally annotated, which can be queried against the specific flavor formation genes using both hidden Markov model alignment and unsupervised Markov clustering. The community composition structure differences can be further characterized using structural variants, such as single nucleotide and codon variants. The challenges due to the compositionality of the generated raw sequencing data are dealt with by using centered-log-ratios leading to inferring the changes in community structure and functions. The implementation of the strategy brought structure to the data analysis and has been used for both DNA and RNA data produced in the project.

WP5 Explorative metagenomics

This work package integrated the findings Work Package 2 & 4, creating models for flavor formation, cheese defects root cause analysis etc. DNA and RNA data from cheese from the production plant and the pilot plant, have been analyzed using the computing pipelines from WP4. In summary, it is clear that the different milk sources and production facilities contribute strongly to the microbial diversity and their specific pathways contributing to flavor/aroma development. Notably, *Streptococcus*, *Clostridia*, and various *Lactobacillus* species differentiate the products and the varied treatments. The Clostridia activity was observed first genetically and thereafter visually in the final cheeses. This means the genetics can be used as an early marker for Clostridium contamination. The eukaryotic viral diversity and abundance have decreased during the cheesemaking process, but significantly more bacterial viruses (phages) were found in the pilot plant.

A more detailed breakdown of the key findings include:

<u>Genomes recovered</u>

Each of the 5 separate experiments (3 Skejby, 2 Kalmar) was run through an *anvio* metagenomics, workflow and a microbial MAG co-assembly was produced for each batch separately. This allowed us to produce so-called MAGs (metagenome assembled genomes) which gave us key insights as follows:

In total we find only 11 different microbes/phages in the datasets, from all of which we recover very complete MAGs. This allows us to perform powerful analyses relating to relative frequency and predicted metabolic activities.

The metagenome datasets contain phages from 3 different strains, namely 2 species of Ceduovirus and one species of Skunavirus. The abundance of these correlates with the overall microbial abundance.

We recover the full genomes of the starter cultures, in particular *Lactococcus spp.* (2 strains; *L. lactis + L. cremoris*) + *Leuconostoc pseudomesenteroides*. Phylogenetic analysis based on 187 single gene clusters shared between the genomes, in comparison to a wide range of reference data (37 strains of *Lactococcus*: 30 for *L. cremoris*, 7 for *L. lactis*) and 101 other reference *Lactococcus* NCBI genomes) confirms that the identical strains are present at both Skejby and Kalmar dairies, consistent with use of the same starter culture. We then performed computational metabolic analyses, and the findings reveal that in comparison to *L. lactis*, *L. cremoris* misses certain metabolic pathways, mainly regarding ornithine, cysteine and lysine biosynthesis, keratan sulfate degradation and a multidrug resistance (efflux pump AbcA).

We also find *Clostridium tyrobutiricum* and *Janthinobacterium spp. (possibly lividus?)* in Skejby sample D12 at low abundance, and *Streptococcus spp. (possibly termophilus?)* from Skejby sample D13 at low abundance.

Process specific insights

With regards to milk pretreatment, the major difference is that the microfiltrated milk clearly contains many fewer microbial genomes and total, thus microbes than the other milk sources, confirming the efficacy of microfiltration.

We observed that although relatively low frequency, *Janthinobacterium* tends to be present in all the raw milk batches, although with notable reduced frequency in the microfiltered milk. This has possible relevance given their known link to milk spoilage. See **Figure 3** as an example.

With regards to the bacteriophages, we can see in the data they are clearly present in the milk upon time of receipt, and that neither microfiltration nor pasteurization fully removes them from the process (although microfiltration reduces their prevalence by approx. 2.3x). Furthermore, we observed their frequency clearly increases in the steps prior to brining, demonstrating their likely interaction with, and reproduction in, the bacteria as they reproduce in the developing cheese. Notably however we see location specific differences in this regard. For example, on average through the ripening process, the Danish milk has 9.7x more phages per *Lactococcus* cell. (average of 1.05 copies in Swedish milk vs 10.14 copies for Danish milk)

With regards to the bacteria present, we find:

i) Overall, the bacterial composition was remarkably stable across all batches, regardless of geographic site or temperature profiles, although there are small exceptions where microbes appear at low frequency in only certain samples.
ii) Although present at low abundance in all samples, *Leuconostoc* is present and stable throughout the whole process.
iii) In contrast, where present (sample D12) *Clostridium* tends to appear towards the end of the ripening process, which may have consequences on the resulting flavour profile.
iv) There is very little difference between the rind and core community.

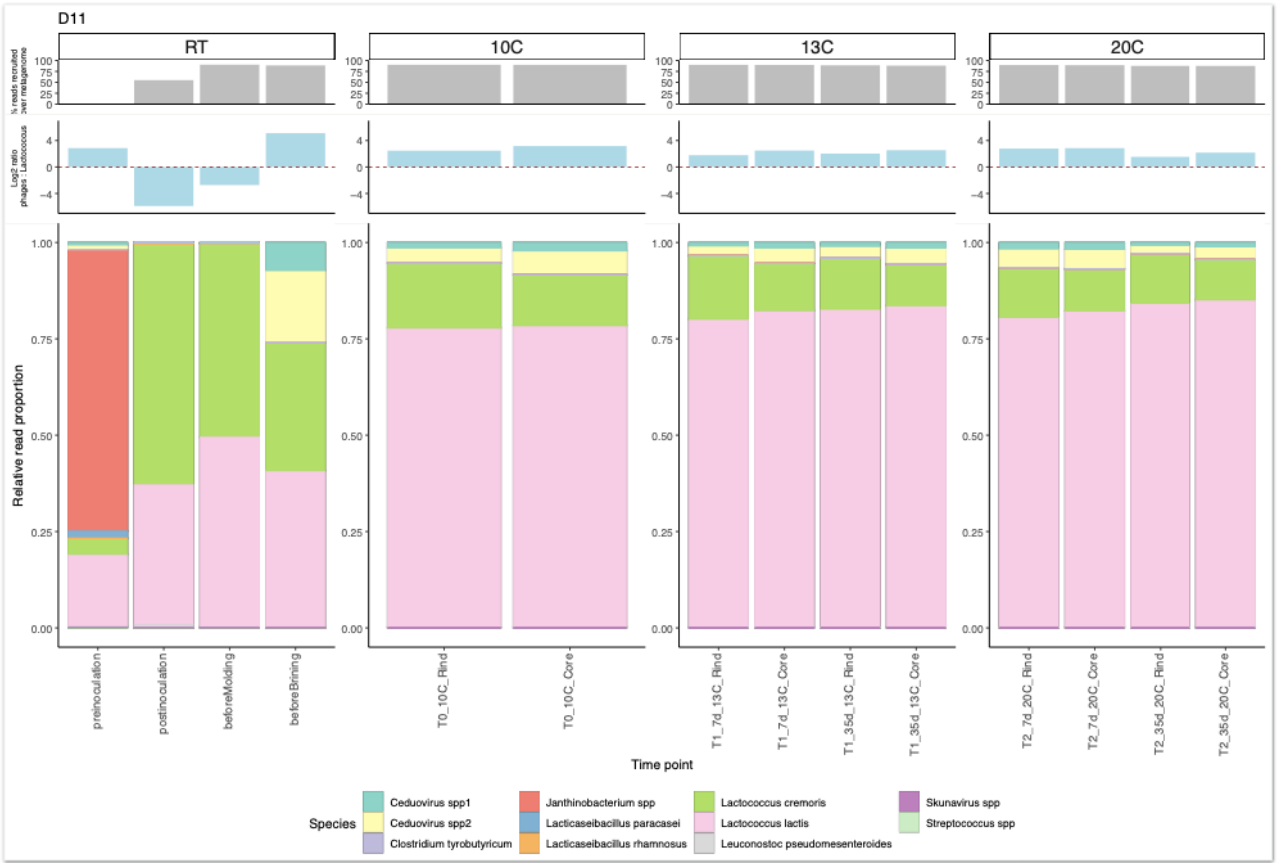


Figure 3. Example of metagenomic insights using Sample D11 (Skejby, microfiltered milk) as a case. Multiple things can be read from the data. I) Data is separated by 4 stages, the room temperature (RT) initial cheese making stages, the initial ripening at 10C, and then the 2 alternate later ripening steps (13C and 20C). Bar charts in the bottom layer show relative abundance of the 11 identified microorganisms at different stages of the process, including if sampled separately for core and rind. The middle blue layer shows the relative abundance of phages to bacteria. The top grey layer is a measure of how much of the data could be identified. Notes of interest include (i) how microfiltration has a major effect on reducing possible spoilage microbe *Janthinobacterium* (red) (ii) how the core and rind communities are very similar in composition, (iii) how during ripening the communities are very similar, despite temperature variation, (iv) although post addition of culture *L. cremoris* is dominant, rapidly *L. lactis* dominates, (v) phages are present in the milk prior to inoculation, and increase in frequency throughout the initial process, likely due to interaction with the microbes added in the culture.

Conclusions

Thanks to the developments made in the laboratory while starting this project, as well as decreases in the cost of data generation, we have managed to generate an enormous amount of data in this project. While exciting, this has come at the cost of enabling us to only scratch its surface so far. In particular, we have not been able to fully explore the metatranscriptomic results – beyond validating that the data is high quality. As such, we anticipate in the upcoming year to both prepare peer review papers on the results of the DNA analyses, but also initiate RNA analyses to tie together the DNA results with the likely functions, thus metabolic data generated. In doing so we anticipate the results will have even larger implications for the industry.

12. The relevance of the results, including relevance for the dairy industry

The MetaCheese project has provided insights into the structure of the microbiome in cheeses produced on the bases of different milk bases and different locations. When applying the same concentrated and complex lactic acid bacteria-based starter solution the microbial community remains stable across all cheeses produces regardless of location, milk base, temperature profile during ripening and the present of phages. However, small variations were identified especially linked to the unit operations applied for the production of milk bases or different cheese vats, showing that a high level of hygiene is still of importance for the dairy industry.

The knowledge gained can help the Danish dairy industry ensure robust production of premium quality cheeses, even when they need to move production to different locations or apply different milk bases. This in turn will serve to increase not only the cost-efficiency of cheese production in general throughout Denmark, but also the overall cheese quality and in the end also benefitting the consumer. Furthermore, the biological insights that will be obtained when the full systematic multi-omics approach have been applied will also provide new insights into the role of the activity of the identified microorganisms during fermentation in general, which will serve to have a wider impact when designing new fermentation solutions for food industry.

The MetaCheese project is also providing insights into the flexibility of modern industrial cheese production and the influences on final cheese quality, by applying the above-described multi-omics approach to explore the structure and functional role of the cheese microbiome. This knowledge will help the Danish dairy industry ensure robust production of premium quality cheeses, even when they need to move production to different locations or apply different milk bases. This in turn will serve to increase not only the cost-efficiency of cheese production in general throughout Denmark, but also the overall cheese quality and in the end also benefitting the consumer. Furthermore, the biological insights obtained from the systematic multi-omics approach will provide new insights into the role of different microorganisms during fermentation in general, which will serve to have a wider impact across areas interested in fermentation but also development of culture-based solutions for the food industry.

By this fast-track analytical toolbox we can identify the structure and function of the cheese microbiome and show its variation across the cheese making process but also as a function of production site, milk bases and ripening temperatures. Such knowledge can be a help to ensure robust production of premium quality cheeses, even when it is needed to move production to different locations or apply different milk bases. This insight can also be used to show the originality of local produced cheeses which is an increased consumer demand.

The principal societal impact will be improved quality and consistency of cheese production. This in turn could lead to reduced costs in cheese making and minimization of waste products. This in turn will both reduce cost of cheese to the end users, and in parallel minimize the climatic impact of both the milk and cheese production itself.

13. Communication and knowledge sharing about the project

[Publications, oral presentations etc. are listed. Please add pdf. files with published papers]

Papers in international journals:

Two in preparation.

(1) Metagenomic based community dynamic shifts during a continental cheese formation because of differences in milk base.
(2) Metatranscriptomics of cheese formation during continental cheesemaking.

Popular science article:

Lene Tranberg Andersen & Tom Gilbert. The challenge of replicable cheese. Mælkeritidende 2021(3). https://maelkeritidende.dk/sites/default/files/udgivelser/Forskningsartikler/mt_04_2021_metacheese.pdf

Lene Tranberg Andersen, Juan Antonio Rodríguez Pérez & Tom Gilbert. The changing cheese microbiome. Mælkeritidende 2023(5): 14-15.
https://maelkeritidende.dk/sites/default/files/udgivelser/Forskningsartikler/sider_fra_maelkeritidende_nr._5_2023_hoej_cheese_microbiome.pdf

Student theses: None

Oral presentations at scientific conferences, symposiums etc.: None

Oral presentations at meetings: Four oral presentations to the DDRF Coordination Group.

14. Contribution to master and PhD education

None

15. New contacts/projects

Chr. Hansen A/S and University of Copenhagen will continue the collaboration to fully explore the metatranscriptomic results and understand the functional role of the cheese microbiome.