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Åsmund Rinnan:

PhageWarn – Forudsigelse af bakteriofag-problemer på ostemejerier

PhageWarn – Prediction of phage problems on cheese factories

Mejeribrugets
ForskningsFond

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Final report

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1. Title of the project

Danish: Forudsigelse af bakteriofagproblemer på ostemejerier (PhageWarn)

English: Prediction of phage problems on cheese factories

2. Project manager

Åsmund Rinnan, associate professor (1/10-2022 to 31/5-2023): aar@food.ku.dk
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3. Other project staff

Dennis S. Nielsen, Professor, University of Copenhagen, Department of Food Science
Søren K. Lillevang, Arla Foods
Louise Tjørnum Kanz, Scientific assistant (01-06-2020 (50% time to 01-12-2020) to 31-01-2022)
Göksen Arik, Post.doc salary financed by Tübitak (01-09-2020 to 28-02-2022). Was hired as post doc for 12 months from 01-03-2022 to 28-02-2023.
Dorentina Homulli (ERASMUS trainee) 01-11-2021 to 31-07-2022. Hired as research assistant from 01-08-2022 to 31-12-2022.
Finn K. Vogensen, Associate Professor Emeritus (01-10-2022 to 31-05-2023).

4. Sources of funding

Danish Dairy Research Foundation
One year post doc from Turkey (Tubitak) for Göksen Arik
6 month post doc from Finland for Paulina Deptula
Salary for three VIPs from University of Copenhagen: Finn, Dennis and Åsmund

5. Project period

Project period with DDRF funding: 01/2020 – 12/2022

Revised, if necessary: 06/2020 – 05/2023

6. Project summary

6.1 Danish:

6.1.1 Fra projektbeskrivelsen

Bakteriofager er stadig den hyppigste årsag til syrningsproblemer i osteproduktionen i Danmark. Dette fører til længere produktionstider, svingende vandprocent og ændringer i smag og tekstur, og det kan i værste fald betyde, at patogene og skadevoldende bakterier kan udvikle sig i osten. Syrningstesten, der i dag anvendes på mejerierne til påvisning af syrningsproblemer, kan ikke forudsige, hvornår problemerne opstår. PhageWarn projektet vil, baseret på kendskabet til de anvendte starterkulturer, udvikle matematiske modeller der, baseret på hurtige analysedata, kan forudsige, hvornår syrningsproblemer opstår. Dette vil give mejerierne mulighed for at gribe ind, inden problemerne opstår.

6.1.2 De vigtigste resultater

I løbet af projektet har vi udviklet et microtiter-baseret screening assay til at følge individuelle starter isolaters sensitivitet til syrningshæmning. Vi har brugt meget tid på at forbedre beskrivelsen af, hvilke typer fager, der er til stede i prøverne. Dette arbejde har fokuseret på at udvikle primere for at detektere specifikke fager i *Lactococcus* via qPCR. Vi har også identificeret nye RBP-gener i lactococcal fag-gruppe 936-fager, og designet primere til disse. Derudover er qPCR-protokollen blevet opdateret, sådan at vi nu kan arbejde med fortyndede valleprøver, fremfor på metavirom-DNA. Vi har også fundet et nyt bakteriofagresistensgen, der ikke tidligere er kendt i LAB, og identificeret ca. 75% af genomet af *Lactococcus*-isolaterne i starterkultur G. Også, nye formodede R/M and mislykkede infektionsmekanismer er identificeret. Vi har desuden identificeret en ny *Lactococcus laudensis*-art i en traditionel dansk starterkultur (starter G), og nye profager i *L. laudensis*. Med en ny tilgang til at modellere data fra ICinac for starterkultur E, ser det ud til at ΔpH/dT er et bedre mål for at bestemme syrningshæmning end tiden til pH 5,5 eller pH efter seks timer. Som det sidste resultat har vi startet en Amplicon-sekvensering ved at bruge Oxford NanoPore-teknologi for RPB fra 936 fag-gruppen som vil blive brugt på valleprøver fra starterkultur E og G efter at PhageWarn er afsluttet.

6.2 English:

6.2.1 Summary taken from the project description:

Bacteriophages are still the most prevalent reason for acidification problems in the cheese production in Denmark. This leads to longer production times, variable water contents, changes in flavor and structure and worst case allow for development of pathogenic and spoilage bacteria in cheese. However, the acidification assay, used today at dairies to detect acidification problems, cannot predict when acidification problems will occur. The PhageWarn project will, based on knowledge of used starter cultures, develop mathematical models that, based on fast analysis data, can predict when acidification problems will appear. This will allow the dairies to make interventions before problems appears.

6.2.2 Highlights of the results from the project:

During the project, we have developed a microtiter-based screening assay to follow the sensitivity of individual starter isolates to acidification inhibition. We have spent considerable time preparing for a more detailed description of phages present in the samples. This work has been focused on developing primers to detect specific pages in *Lactococcus* via qPCR. We have also identified new RBP genes in the lactococcal phage group 936 phages, and designed primers for these. Furthermore, the qPCR protocol has been modified, so that we can work with diluted whey samples instead of metavirome DNA. We have also found a new bacteriophage resistance not previously found in LAB, and identified approx. 75 % of genomes of *Lactococcus* isolates in starter G. Also, putative new R/M and abortive infection mechanism have been identified. Further, we have identified new *Lactococcus laudensis* species in a traditional Danish starter culture (starter G), as well as new types of prophages in *L. laudensis*. Through a novel approach to model ICinac data from starter culture E, it seems like ΔpH/dT is a better measure of acidification delay than time to pH 5.5 or pH

after 6 hours. However, for this to work, it is necessary with a continuous monitoring of the pH during acidification. As a final result we have initiated Amplicon sequencing using Oxford NanoPore technology for RPB from the 936 phage group and will be further analyzed on whey samples from starter E and G after the project has been terminated.

7. Project aim

7.1 Danish:

Formålet med projektet er at udvikle matematiske modeller for at prædiktere, hvornår det er sandsynligt, at der opstår bakteriofag-problemer med *Lactococcus lactis* og *Leuconostoc*. Modellerne vil være baseret på modelsystemer, der bliver etableret i laboratoriet, med udvalgte starterkulturer, og testet hos udvalgte mejerier, hvor disse starterkulturer anvendes. Modellen baseres på metavirom-sekvensering, high-throughput-qPCR, og syrningskurver. En implementering af denne type model vil være fordelagtigt for mejerier, der anvender udefinerede mesofile starterkulturer ved at sikre at en kontrolleret fermentering, effektivt ressourceforbrug og konsistent produktion af ost med høj kvalitet, og samtidigt forhindre tab.

7.2 English:

The aim of the project is to develop mathematical models for predicting when phage problems with *Lactococcus lactis* and *Leuconostoc* phages are likely to appear. The models will be based on laboratory simulations with selected starter cultures and tested on selected dairies that are using these starters. The model will be based on metavirome sequence data, high-throughput-qPCR data, as well as acidification delay data. Implementation of such a model would benefit the dairies using undefined mesophilic starters by ensuring optimal fermentation control, efficient use of resources and consistent production of high-quality cheeses, and at the same time prevent losses.

8. Background for the project

Previously, we in the FTP-financed project MetaPhageLAB (running 2012 to 2016) studied phage development at five Danish and Swedish cheese factories. For these investigations, we developed High-Throughput qPCR assays[1] that were able to detect phage genomes within the most common *lactococcal* and *Leuconostoc* phages. The detection limit for each phage species were at levels from 10^3-10^{10} phage genomes per g of whey or bulk starter culture. At the five dairies the level of the most common lactococcal 936 phages species varied between 10^6 to 10^{10} and in whey samples from 10^4 to 10^9. For the P335 phage species, the level in bulk starter cultures were typically constantly at 10^6 to 10^7 dependent on the starter culture used. Also, in most cases a 100-fold lower level was found in whey. This indicated that the P335 phages present most probably were due to spontaneous induction of prophages from the chromosomes of the lactococcal starter strains[2]. The broad-host range lactococcal c2 phage species were found in low numbers now and then in bulk cultures and in whey, and occasionally a >100-fold increase was seen in the cheese vat within the first 2 hours of production. *Leuconostoc* phages species were also present in varying concentrations dependent on the dairy, but almost always at a 10-fold lower number than the 936 species. One of the main findings was that there was no correlation between numbers of phage genomes and acidification problems. Over the last two decades we and others have identified the gene on the lactococcal and *Leuconostoc* phages that codes for the receptor binding protein (RBP-gene)[3-6]. We have shown that there is a correlation between host-range and the amino acid sequence and the host-range[6,7], and thereby also between the RBP-gene and the host-range. After sequencing of approx. 100 genomes of different phage from the 936 species and adding another 100 from public databases we could divide the RBP-genes into 19 RBP types that could be correlated to their host-range. Similarly, we have so far identified 6 RBP types in Leuconostoc phages that we can correlate to host range. In the MetaPhageLAB we also developed a metavirome purification protocol for dairy products that allowed us to isolate all phages present in the raw milk, the bulk starter and whey samples[8]. If the number of phage genomes were higher than 10^7 in 20 mL of sample, we were able to sequence all the

phages present but only in pieces of 3-400 bp (out of 20.000-40.000 bp) and because many of the phages are very similar it is not presently possible to assemble whole phage genomes from metaviromes from dairy samples. However, we could use the obtained sequences to map to the RBP-genes, and thereby get a relative proportion of each of the recognized RBP-gene types related to hosts attacked in the starter culture[1]. When we compared these data with acidification problems it clearly indicated that it is not the total number of phages present that is important but the total number of diverse RBP and then total number that appears to be important for acidification problems. This led us to propose the present PhageWarn project to model the relationship between RBP diversity and total number of phages in a mathematical model. One other observation from those dairies that used rotation between starters was that when they changed the starter from A to B it took only 1-2 days before the RBP-type related to starter A almost disappeared while the RBP-type that belonged to starter B was present in the environment and came back within 1-3 days. And then vise-versa when they changed back to starter A. We saw this on two dairies and could identify some design flaws on both dairies. We saw similar observations in one very large dairy in a pre-project to MetaPhageLAB in Denmark, and here the company knew of the design problem. In such dairies, the PhageWarn project may not give sufficient warning, but may be a tool to track where the surviving phages are located. Another outcome of MetaPhageLAB project was that metavirome sequencing is to slow using the present technology (3-4 days), while HT-qPCR may give data within 24 hours after a sample has been received and until data is available.

8.1 Literature references:

1. Muhammed, M. K. et al. (2017) PLoS One 12:e0174223.
2. Muhammed, M.K. et AL. (2018) Int. J. Food Microbiol., 272: 61-72
3. Stuer-Lauridsen, B. et al. (2003) Virology, 309: 10-17.
4. Dupont, K. et al. (2014) Appl. Environ. Microbiol., 70: 5818-5824.
5. Vegge, C.S. et al. (2006) J. Bacteriol., 188: 55-63.
6. Kot, W. et al. (2013) Applied and Environmental Microbiology, 79: 3311-3314.
7. Dupont, K., Vogensen F.K., and Josephsen J. (2005) J. Appl. Microbiol 2005, 98, 1001-1009
8. Muhammed, M. K. et al. (2017) Appl. Environ. Microbiol. 83:e00888-17

9. Deviations

9.1 Scientific

During the project it became evident that the existing primers for the RBP genes - qPCR were not sufficient. Therefore, considerable time in the project has been focused on developing novel primers for the detection of the different bacteriophages rather than getting specific phage data from the acidification samples. Furthermore, it has been necessary to work heavily on speeding up the metavirome isolation.

Our freezer broke down during the project, and several samples were lost. Unfortunately, we also lost starter A in this process, and we were unable to obtain the bulk starter culture again, even though we asked two dairies for it. We therefore decided to leave it out of the project.

Problems with metavirome DNA isolation and stability meant that the necessary analysis could not be reached sufficiently early, so that the initial mathematical models could be tested during the project period. When we finally solved this problem, it was too late to analyze all data, and we also ran out of money. This also means that we have not been able to create a prediction model based on data from high-throughput qPCR or HTS. We do, though, have a model for investigation of deviations of the acidification curve (see below), but did not have time to test this model in a dairy setting. We do have all samples in the freezer so it is possible to analyze data after the project, if we can finance it from other sources. Finn K Vogensen has other sources that will be used for this purpose.

9.2 Financial

Funding for the project came late (June 2020). So, the start of the project was delayed almost one year.

9.3 Timetable

The timetable has been adjusted due to staffing and financing issues. Also, Covid-19 has in periods been an obstacle for working in the laboratory.

The ICinac necessary for WP2 only arrived in beginning of December 2020, therefore we were more than half a year late in setting up the phage accumulation assays.

10. Project results

10.1 WP1: Expand library of and characterize Lactococcus and Leuconostoc RBP genes

This work has been initiated. Approx. 200 isolates from each of two bulk starter cultures (E and G) have been purified and frozen. Whey samples have been obtained from two dairies using the two starter cultures. A third culture (A) and whey samples were obtained from a Danish dairy, but unfortunately a freezer breakdown meant that the starter culture was lost. Attempts to obtain the bulk starter culture A and whey samples from two different dairies was unsuccessful.

Approx. 150 new RPB genes have been added to our library of lactococcal RBP genes. These are based on publicly available sequences as well as own new phage sequences. We have also found additional three new RBP gene groups from Leuconostoc phages. Primer design for lactococcal phage species (949 and P087 phage groups) have been developed and primer design for new lactococcal phage 936 RBP groups have been developed, and primers for leuconostoc phage RBP genes are under way.

We have had problems isolating sufficient DNA for metavirome sequencing, and also, we have seen stability problems with isolated metavirome DNA. This has hampered metavirome sequencing to evaluate diversity of phages belonging to 936 phage genera and Leuconostoc phages. It appears that at least 10^7 phages particles per mL of whey is necessary for sufficient DNA, and this has been a problem particularly for starter G but also for starter E as the phage titer was too low in the whey samples we obtained. Stability problems were due to wrong storage conditions (-20 C). which was not realized until late in the project. We still have all whey samples in the freezer at -60 C so it is possible to re-extract metaviromes for sequencing and HTP-qPCR. Late in the project we showed that for qPCR (and amplicon sequencing) we do not need to isolate metavirome DNA, but we can use diluted whey samples that is preheated for 20 min at 95 C before the qPCR is set up. Without dilution we see strong qPCR inhibition.

We are also re-thinking our procedure to see diversity of so far 936 phage species. We are developing primers for amplification of RBP genes directly from diluted whey samples followed by amplicon sequencing using Oxford NanoPore technology.

10.2 WP2: Analyze phage development on different starters in a laboratory environment

Originally three starter cultures were selected for the analysis. The ICinac equipment needed to analyze the acidification development has been acquired, and we have so far used it to analyze data from phage accumulation experiments over 5 days with 10 different whey samples (6 belonging to starter E and 4 belonging to start G) where we simulated high day-to-day contamination (1 %). This has been repeated twice. Our hypothesis was that we would see increasing acidification delays due to phages during the accumulation experiments. This hypothesis was correct for starter E, where it was seen that acidification delays were increasing over time, see Figure 1. To our surprise this was not the case for starter G. Here, we saw acidification delay only from some whey samples the first day, but on the following days no inhibition was seen. We presented the data from the first experiment series on the last DDFF meeting but have since repeated the experiments with similar results. For starter E we have also repeated twice with 1 %, 0.1 %, 0.01 % to simulate lover contamination (down to 1.5 L whey left between fillings in 15.000 L cheese tank). It is

clearly seen that lower contamination reduces the acidification delay the first days but after three propagations (comparable to cheese vat fillings) the acidification delay is equal or higher than with 1 % contamination.

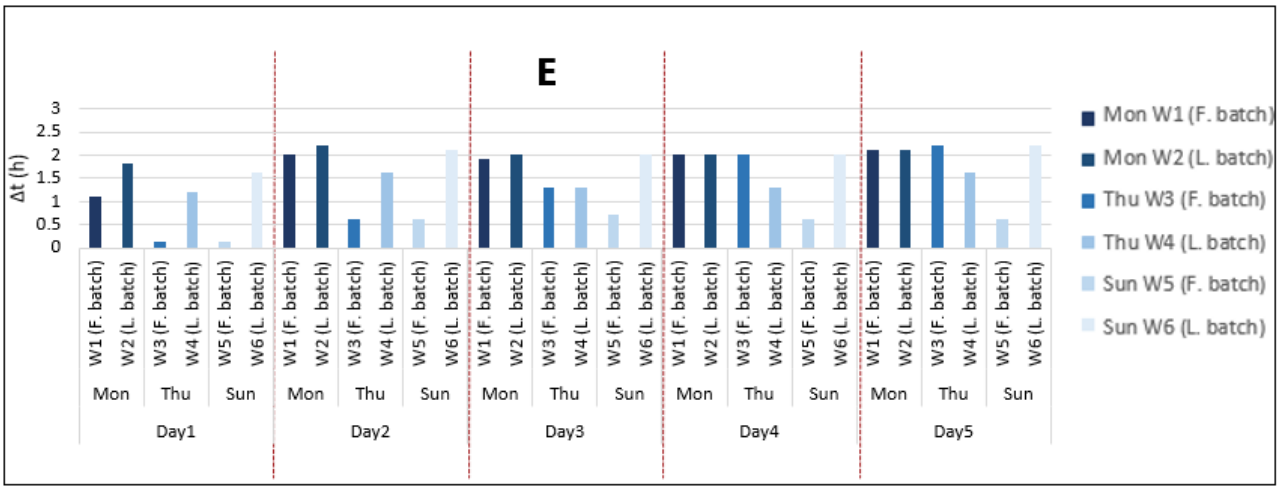

Figure 1: Acidification delays on the different batches of starter culture E during amplification for five days.

We have also developed a microtiter plate-based screening assay for detection of acidification delays from whey samples against individual starter isolates. This assay has been tested on approx. ½ of bacterial isolates from starter E toward the 6 whey samples from starter E (Figure 2), and towards 62 genome sequenced isolates of starter G.

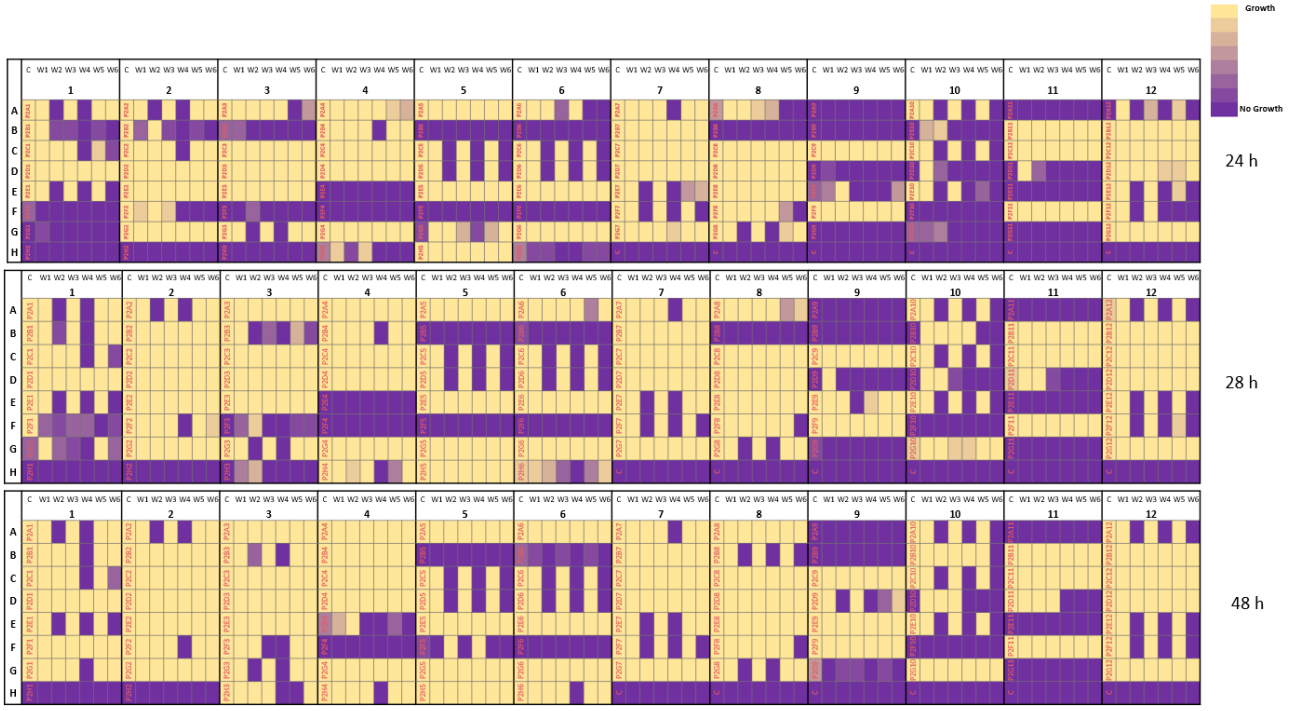

Figure 2: Phage inhibition assay for single isolates from starter culture E with measuring time along the rows.

Randomly chosen draft whole genomes of 51 lactococcal and 12 Leuconostoc starter isolates from starter culture G were obtained from the "Metapredict" project now followed up with the DDRF project "Cassandra". Surprisingly, 9 of the sequenced lactococcal isolates belonged to the species *Lactococcus laudensis*. This species has previously been isolated from raw cow's milk in Italy, but also in a traditional Dutch DL-starter culture "Ur" and it was not found in starter culture E. In 5 of these strains from starter culture G, we have identified and induced new types of prophages,

but have so far not been able to find indicator bacteria for them. They belong to two different prophage species, 4 were related and only one was almost unrelated, the four of them had a very long spike that broke of in CsCl gradients, and therefore were no longer infectious.

However, when we also analyzed the sequences from 63 isolates in starter culture G for genes probably involved in phage resistance mechanisms, we surprisingly found at least one mechanism not previously found in Lactic Acid Bacteria. The genomes of approx. 75 % of all investigated lactococcal genomes had genes for this new mechanism. If the genes are functional then this may explain the unexpected findings for starter G. But it requires further research. Also, several other abortive and restriction-modification systems were found in the genomes. We are presently trying to identify the putative bacterial phage receptor genes. We expect that this will allow us to use amplicon sequencing of receptor genes to evaluate which strains are sensitive during infection (again using Oxford NanoPore technology).

Today, all 200 isolates from starter culture E and G have (after the PhageWarn project ended) been genome sequenced in the DDRF-project Cassandra, and the data showed that the two starter cultures were fundamentally different on species level as well as strain level.

10.3 WP3: Develop a prediction model for early warning and testing the prediction model on selected dairies.

We have continued with analyzing data from one dairy from the previous MetaPhageLAB using multivariate data analysis, but we realized through that we needed information both of phage diversity and numbers present but also on the strain diversity to generate a good model. We tried during the research project to also develop methods to determine starter strain diversity, but this goal has not been reached.

We have analyzed the ICinac model experiments using different modeling systems with a focus on using as much of the data as possible. Through proper pre-processing of the data by subtracting the control, and investigate the 1st derivative of these deviations we were able to separate the measured samples according to the contamination level (0.01, 0.1 or 1%, see above, and Figure 3), the used whey (three different ones), as well as how many times the contamination was repeated (5 days in total). This approach seems promising in extracting yet more understanding from the acidification curves than only recording the delay time to a specific pH or a difference in pH at a specific time. We are still investigating the possibility of expanding this approach to get an even deeper understanding of the behavior of these curves.

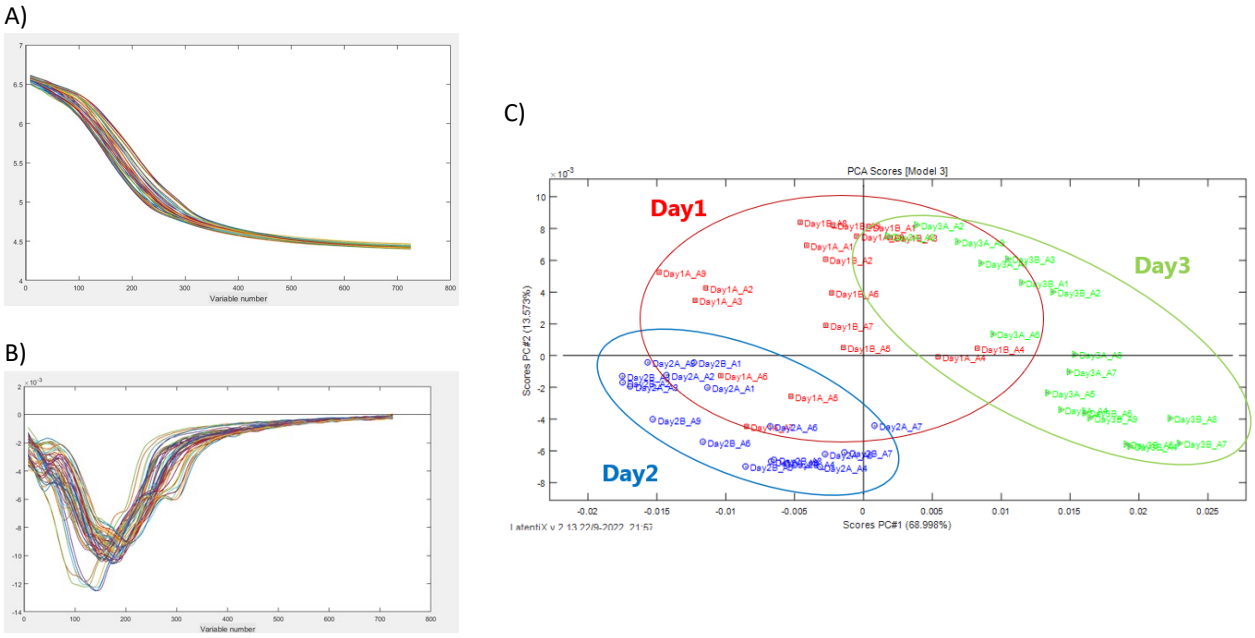

Figure 3: Results from acidification experiments on starter culture E during amplification. A) The raw acidification curves, with pH on the y-axis and time on the x-axis, B) the ΔpH on the y-axis and time on the x-axis (i.e. the derivative of the pH), and C) the corresponding score plot indicating a clear change in the acidification pattern according to the amplification.

10.4 Summary

- A microtiter-based screening assay has been developed to follow starter individual isolates sensitivity to acidification inhibition.
- Two primer sets have been developed to detect *Lactococcus* phages belonging to the 949, and P087 group of phages and they can be detected by qPCR.
- New RBP genes have been identified in the lactococcal phage group 936 phages. And primer sets have been designed but not yet been confirmed to work.
- A new bacteriophage resistance not previously been found in LAB has been identified in approx. 75 % of genomes of *Lactococcus* isolates in starter G. Also, putative new R/M and abortive infection mechanism have been identified.
- We have modified our qPCR protocol, so that we can work with diluted whey samples instead of metavirome DNA.
- New *Lactococcus laudensis* species identified in a traditional Danish starter culture (starter G).
- New types of prophages identified in *L. laudensis*. A manuscript is under preparation.
- Modelling of ICinac data from starter culture E. It looks like delta-pH/dT is a better measure of acidification delay than time to pH 5.5 or pH after 6 hours. This requires continuous monitoring using e.g. ICinac.
- Amplicon sequencing using Oxford NanoPore technology has been initiated for RPB from the 936 phage group and will be further analyzed on whey samples from starter E and G after the project has been terminated.

10.5 Project goals

Relative to goal 1, goal 2, etc., please briefly respond to the following questions:

- WP1: Most goals have been accomplished concerning sequencing of new phages. Also, we have developed a qPCR technology so that we can quantify phages directly from whey samples without the costly and time-

consuming metavirome DNA isolation. The developed method also allows us to conduct amplicon sequencing over genes several kilobases long

- WP2: The model experiments have been conducted for 2 out of 3 starter cultures using ICinac equipment. Due to freezer failure the third planned starter culture died (lost 90 % of cells) over a Christmas. New methods to follow phage diversity have been developed and will be used to analyze frozen whey and starter samples after termination of the project.
- WP3: The first simple models based only on acidification assay has been conducted and will be further analyzed when amplicon sequencing data and HT-qPCR data is ready.

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

11.1 Scientific perspectives:

The scientific perspective is still to gain more knowledge on phage-host interactions by studying how phage develop against undefined mesophilic starter cultures. We did not reach this goal in the present project, but we developed new methods to faster evaluate phage content by qPCR in whey samples without having to isolate metaviromes. A DNase treatment of the whey sample to remove bacterial genomic DNA, followed by a simple dilution of whey sample that was then heat-treated for 20 min at 95C was sufficient to release phage DNA for qPCR. This method was developed late in the project and was not used for all the samples due to time limitations.

Also, we developed new qPCR assays that will target the phage groups P087 and 949, increasingly isolated from whey samples. We are therefore able to quantify them in whey samples for future research.

Also, we added approx. 150 new sequences from literature and own sequencing of the 936 phage group, so that we have approx. 350 phage genomes available and thereby their Receptor Binding Protein (RBP) genes. We have designed new primers to new groups of RBP genes, but they have not yet been completely evaluated by qPCR. Similarly, we have identified three new RBP groups from literature and are in the process of designing primers for these for qPCR. We can use them for further research on the whey samples.

We have conducted ICinac experiments with starter E where we as expected increased the acidification problem. This was done with 6 whey samples that was propagated 5 times with 1 % inoculum from previous fermentation and then later for 3 whey samples with 0.1 %, 0.01% and 0.001 %, which showed similar results but delayed up to 3 days.

When we did same type of experiments with starter culture G we saw a completely different picture. After one propagation we saw that the culture G regained its acidification power using 1 % inoculum of whey. This was a big surprise as we had not expected this. When we later got genome data from the Arla Project “MetaPredict” we realized that many of the strains had a new type of phage resistance mechanism not previously found in LAB. We have recently got access to the genome sequences of all the 400 isolates from the DDRF-Cassandra project, and presently evaluation the phage resistance mechanism found in isolates of the two starters. This will be conducted in the Cassandra project.

Due to problems with metavirome isolation and later stability problems of isolated phage DNA, we spend a lot of time to optimize metavirome isolation to reduce time for isolating the DNA. We realized that the different fast methods did not work as we got to little phage DNA to be able to sequence the metavirome. Also, we had problems of stability of the DNA with the new fast isolation methods. We planned to use 24 x qPCR assays vs. 192 samples HT-qPCR, but the DNA disappeared within one week at -20C. So, although we had the assays, we newer used them because of the stability problems. When we finally found out that we could do without metavirome DNA it was to late to do the experiments and we also ran out of money. The whey samples will be reanalyzed after this project is finished with other financing.

The mathematic model based on HT-qPCR was not developed for started E as expected. Instead, a model passed on ∆ph/dT was developed. This model seems to be better predicting the future pH delay than time to pH 5.5 or pH after 6 hours that is used today, but it requires continuous pH measurements using equipment like ICinac.

When data from HT-qPCR and amplicon sequencing is available we will look into developing a mathematical model for predicting acidification problems.

11.2 Social perspectives:

Now there is no social perspectives. If we later are able to build a convincing model, then of course there will be social perspectives, with less stress on the dairy workers because acidification problems can be foreseen, and therefore can be avoided by either changing cleaning and disinfection regime or changing the starter cultures.

11.3 Dairy industry perspectives:

The main perspective for the dairy industry is that ΔpH/dT gives a better description of acidification delays than ΔpH at pH 5.5 and pH after 6 hours. Also, a number of primer sets have been designed that can detect total number of phages belonging to the lactococcal phage groups 936, c2, P335, P087, and 949. In addition, we have designed primer sets for quantification *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides* phages either together or individually. We have developed primer sets targeting receptor binding protein genes for the 936 phage group and for the Leuconostoc phage groups. We have also found that we ca avoid the time consuming metavirome DNA isolation from whey for qPCR by diluting whey samples.

12. Communication and knowledge sharing about the project

Papers in international journals:

Wenwen Li, Axel Soto-Serrano, Farhad M. Panah, Göksen Arik, Paulina Deptula, Daniella Lucena, Finn K. Vogensen, Lukasz Krych, David A. Baltrus. Draft genome sequences of 53 Lactococcus and Leuconostoc strains isolated from two undefined DL-starter cultures. Microbiology Resource Announcements, Volume 13, Issue 7, 2024, https://doi.org/10.1128/mra.00228-24.

Easily read papers:

L. Kanz, F.K. Vogensen, D.S. Nielsen, & Å. Rinnan (2020) Varslingssystem skal forudsige syrningsproblemer forårsaget af bakteriofager. https://maelkeritidende.dk/forskningsartikler/10-2020

Student theses:

Yuandong Sha (2022) Dairy Phage Warn Project 15P, PUK project thesis

Yuandong Sha (2022) Development of phage on an undefined DL culture and analysing the phage strain diversity and the phage diversity attaching the Culture. Master thesis, 30 ECTS

Oral presentations at scientific conferences, symposiums etc.:

Other:

Arik et al. (2022) Characterization of *Lactococcus laudensis* prophages by whole genome sequencing. (Poster at Viruses of Microbes, July 2022, Portugal).

Arik et al. (2022) Characterization of *Lactococcus laudensis* prophages by whole genome sequencing. (Poster at Oxford Phage Symposium, September 2022, UK).

13. Contribution to master and PhD education

Yuandong Sha, MSc, December 2022.

Dorentina Homulli (ERASMUS trainee) 01-11-2021 to 31-07-2022. Hired as research assistant from 01-08-2022 to 31-12-2022.

14. New contacts/projects

Associate Professor Rinnan has been part of two larger applications together with Søren Lillevang as a result of the contact initiated through this project. For one of the projects the contract is being negotiated, while the other we are awaiting the final evaluation. Emeritus Associate Professor Finn K. Vogensen is together with Assistant Professor Paulina Deptula associated to the DDRF-Cassandra project led by Associate Professor Lukasz Krych. Here we will look into details on phage resistance and also phage propagated on the starters E and G. FKV will also participate in selection of strains for fermentation experiments.