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Susanne Knøchel:

AFunDay – Antifungal biobeskyttelse af mejeriprodukter

AFunDay – Antifungal Dairy Product Bioprotection

Mejeribrugets
ForskningsFond

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Final report

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

1. Title of the project

Antifungal biobeskyttelse af mejeriprodukter
Antifungal Dairy Product Bioprotection.

Acronym**: "AFunDay**"

2. Project manager

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4. Sources of funding

Total budget: 4,175,475 DKK.

Financed by The Milk Levy Fund 1,973,674 DKK, Arla Foods 480,000 DKK (210,000 DKK, in-kind), Sacco 105,000 DKK (in-kind) and UCPH has co-financed the remaining 1,616,801 DKK.

5. Project period

Project period with DDRF funding: January 2019 – December 2020

Revised, if necessary: The period was extended to the end of 2022 due to the COVID-19 pandemic.

6. Project summary

In Danish:

Formål: Projektet havde til formål at optimere brugen af biobeskyttende kulturer til at hæmme gær- og skimmelvækst i fermenterede mejeriprodukter som yoghurt og skyr ved at belyse interaktionen mellem potentielle biobeskyttende kulturer, de forskellige fordærvelsesorganismer, anvendte starterkulturer og specifikke matricer. En bedre forståelse af disse sammenhænge vil facilitere både søgning efter antifungale kulturer og den målrettede anvendelse af disse i forhold til produkt- og problemkarakteristika. Det har derfor også været et formål at samle og karakterisere en række hhv. gær og skimmel til brug for videre arbejde inden for området.

Resultater: Der er blevet indsamlet gær* og skimmelsvampe fra relevante produkttyper. To hurtigmetoder afprøvet i industrien til typning af gær (hhv. FTIR og MALDI-TOF MS) er blevet sammenlignet med en anerkendt standardmetode (sekventering af 26S rRNA genet (D1/D2 regionen)) samt en nyere nanopore-baseret sekventeringsteknologi (ON-rep-seq). Både MALDI-TOF og ON-rep-seq vurderes at kunne anvendes til at sammenligne isolater og til identifikationsformål, specielt når databaserne bliver bedre udbyggede, mens FTIR havde for stor støjfølsomhed. På baggrund af isolaternes karakteristika, herunder følsomhed over for de afprøvede biobeskyttende kulturer, er der udvalgt et panel af hhv. 13 skimmel og 11 gær, der kan stilles til rådighed for videre forskning og udvikling. Potentielt biobeskyttende stammer (modtaget fra Sacco) er blevet undersøgt. Forskellige antifungale mekanismer er blevet beskrevet og afrapporteret. Der sås kun meget beskeden hæmning ved brug af cellefri filtrater og endnu mindre ved brug af de flygtige komponenter. Blandt de undersøgte stammer med størst antifungal effekt var hovedmekanismen næringsstofkonkurrence om mangan, der kun findes i meget lave mængder i rent mælkebaserede produkter. Vækstinhibering kunne således modvirkes ved tilførsel af mangan. Af skimmelkulturerne var *Penicillium* (bortset fra *P. roquefortii*) mere følsomme end *Mucor* spp., og generelt var skimmelkulturerne betydeligt mere påvirkede

end de afprøvede gær. Ved 25 grader eller ved højt startniveau af gær sås ingen hæmning af gær. Tilsætning af biobeskyttende kulturer fremskyndede generelt syrningen.

* Udvalgte stammer er allerede indgået i et parallelt MFF-støttet projekt ”Improve Dairy Life” og nogle vil indgå i et nystartet studenterprojekt med dansk industrideltagelse.

Konklusion: Et panel af skimmel- og gærsvampe med forskellig følsomhed over for de testede, biobeskyttende mælkesyrebakterier er samlet og til rådighed for interesserede. Metoder til typning af gær er evalueret. Antifungal effekt af kulturer, filtrater og flygtige stoffer er undersøgt, hvor den primære hæmningsmekanisme i yoghurt viste sig at være konkurrence om mangan. Skimmel var mere påvirket end gær, hvoraf en del var ufølsomme. Ved høj temperatur eller forureningsgrad sås ingen hæmning af følsomme gær. Der er indtil nu udarbejdet fem videnskabelige peer-reviewed artikler, en ph.d.-afhandling, fire speciale-/bachelorrapporter i samt en projektrapport forbindelse med projektet.

In English:

Aim: The project aims to optimize the use of bioprotective cultures to counteract growth of molds and yeasts in freshly fermented products such as yoghurt and skyr by elucidating the interplay between the cultures, the spoilage organisms, the starter cultures, and the specific matrices. A better understanding will facilitate the search for better bioprotective cultures as well as the targeted use of these in relation to specific products or problems. It has therefore also been a wish to assemble a panel of characterized molds and yeasts for future testing.

Results: Yeasts* and molds have been collected from relevant product types. Two rapid methods used in industry for yeast typing (FTIR and MALDI-TOF MS) have been compared with an acknowledged standard method (sequencing of the 26S rRNA gene (D1/D2 region)) and a newer nanopore-based sequencing technology (ON-rep-seq). Both MALDI-TOF and ON-rep-seq were considered suitable for comparing isolates and for identification purposes, especially as the databases improve, while FTIR exhibited “noise” problems. Based on the characteristics of the isolates, including sensitivity to the tested bio-protective cultures, a panel of 13 molds and 11 yeasts has been selected for further research and development. Potentially bioprotective strains (received from Sacco) have been examined. Various antifungal mechanisms have been described and reported. Only very modest inhibition was observed using cell-free filtrates and even less with volatile components. Among the tested strains with the greatest antifungal effect, the main mechanism was nutrient competition for manganese, which is only present in very low amounts in pure milk-based products. Growth inhibition could thus be counteracted by the addition of manganese. Among the mold cultures, Penicillium (except *P. roquefortii*) was more sensitive than Mucor spp., and in general, mold cultures were more affected than the tested yeasts. At 25 degrees or at a high initial yeast level, no inhibition of yeast was observed. The addition of bio-protective cultures generally accelerated acidification.

*Selected strains have already been used in a parallel project "Improve Dairy Life" and others will be part of a new student project in collaboration with a Danish industry partner.

Conclusion: A panel of molds and yeasts with different sensitivities towards the tested bioprotective lactic acid bacteria (LAB) cultures is now available. Typing methods for yeasts were evaluated. The antifungal effect of cultures, filtrates, and volatiles were tested and the primary inhibition mechanism in yoghurt was shown to be competition for manganese. Molds were more susceptible than yeast, some of which were

insensitive. At high temperatures or contamination levels none of the yeasts were inhibited. The project has until now resulted in five peer reviewed papers, 1 PhD thesis, 4 Master/Bachelor theses, and 1 project report.

7. Project aim (original)

The overall **aim** is to optimize the use of natural bioprotection in order to inhibit fungal spoilage of fermented, fresh dairy products thereby increasing potential shelf life and export possibilities and avoiding food waste and economic losses. The specific aims of the project are to 1) to create and characterize a collection of spoilage yeasts and molds isolated from the relevant products which, together with a starter culture panel, can be used for future testing, 2) identify major antifungal microbial metabolites from bioprotective cultures 3) characterize their effect on growth of spoilage associated fungi as well as starters in defined systems and relevant products, 4) describe the qualitative and quantitative changes in cell physiology and morphology of selected strains 5) investigate the response of selected target strains at the transcriptomic level and 6) disseminate the knowledge to the Danish dairy industry and the scientific community.

Due to recognition of the role of nutrient competition in the inhibition, some of the subobjectives were changed in the course of the project to include more on the role of manganese.

8. Background for the project

State of the art: Spoilage caused by molds or yeasts is a major problem in the dairy industry. Growth often occurs rapidly after a package has been opened creating access to external contamination, but spoilage organisms may also derive from e.g. added jams or contaminated equipment. Recently, some microbial species suggested for biological control in organic agriculture have been associated with fruit yogurt spoilage (Wrent et al. 2015). Apart from the obvious sensory problems, some contaminating fungi may also be potentially harmful producing mycotoxins. Among the filamentous fungi, various *Penicillium* spp. but also *Aspergillus* spp., *Mucor* spp., *Geotrichum* spp., *Cladosporium* spp. and *Fusarium* spp. are known spoilers of fermented dairy products (Frisvad et al., 2007; Pitt and Hocking, 2009) and also several yeasts are associated with spoilage of dairy products including *Rhodotorula* spp. (mainly *R. mucilaginosa*)*, Debarymyces hansenii*, *Candida* spp., *Kluyveromyces* spp., *Pichia* spp., *Yarrowia lipolytica* (Deak, 2008; Fleet, 1990; Pitt and Hocking, 2009) and species such as *Meyerozyma spp.* (Wrent et al 2015). While there are "golden standards" for typing yeasts, these are often not used in industry where rapid methods with less hands-on time are preferred and although they may provide fast answers there is some uncertainty regarding the capability to differentiate and type strains correctly which may make it more difficult to identify problematic/recurrent strains.

A promising tool for controlling fungal growth is the use of microorganisms producing antifungal compounds or exhibiting other antifungal mechanisms. It has long been known that fungi and other microorganisms may exhibit mutually antagonistic behavior (Axel et al. 2016; Delavenne et al.2013) and that certain yeasts and lactic acid bacteria (LAB) produce metabolites that are able to inhibit fungal growth in dairy products (Magnusson et al. 2003; Wang et al. 2011, Aunsbjerg et al. 2015a). However, a more targeted use of microorganisms and metabolites has been challenged by our limited understanding of the complexity of

many fermented products including the interplay between beneficial starter and non-starter cultures and the undesirable microorganisms. The antifungal microorganisms or metabolites must live up to a number of requirements. They should of course be safe for consumers, but they should also inhibit the undesirable fungi without impairing the sensory attributes of the product and they should not obstruct the growth or functionality of the starter cultures.

Although our understanding of the interactions has improved due to molecular based studies of community biodiversity and dynamics (Wolfe et al. 2014), and the use of metabolic footprinting methods (Honore et al. 2016) and defined model systems (Aunsbjerg et al. 2015b) for detection of novel antifungal compounds etc., there is still a lack of understanding regarding the mechanisms behind the antifungal effects and why various molds and yeasts differ in their susceptibility. Many lactic acid bacteria have specific requirements for nutrients such as manganese, which are only found in low amounts in milk-based products. A paper published during the project found that competition for manganese was important for an observed antifungal effect of a lactic acid bacterial culture (Siedler et al. 2020) and it was therefore obvious to test the importance of this mechanism in the potentially bioprotective cultures examined in the project.

9. Sub-activities in the entire project period

Task 1. The involvement of dairy and starter culture companies should ensure that relevant strains (bioprotective cultures, starter cultures and target spoilage organisms) were being used in the proposed project. Potentially bioprotective strains were provided by SACCO while spoilage yeast and fungi isolated from relevant products were received from various sources incl. SACCO, Arla Foods and ISI Food Protection. The strains were typed and characterized. In the case of yeast, this included comparison of different rapid typing methods applied in industry with standard best practice based on 26S rRNA D1/D2 as well as a more novel ON-Rep-seq sequencing (nanopore technology).

Task 2. The inhibitive effect of the various bioprotective lactic acid bacteria as well as single or mixed non-volatile and volatile antifungals was tested on molds using different assays and specific changes at the cellular level were described.

Task 3. Efficacy of selected cultures was tested in yogurt/yogurt serum matrices and the role of manganese was investigated.

Task 4. Based on the characteristics, including sensitivity or resistance of the spoilers towards the tested bioprotective cultures, a panel of selected test strains (yeasts and molds) was suggested.

Task 5. Dissemination. Scientific publications.

Task 6. Education and supervision of students.

10. Deviations

Scientific deviations: Based on information obtained during the project, more emphasis was placed on the role of nutrient depletion in the last part of the project.

Timetable: Due to the COVID restrictions (both at partner institutions who were responsible for starter culture production and yogurt production and at the university), the experimental work was significantly delayed. The project also experienced some problems in getting laboratory disposables due to the extensive COVID testing (Just during the first year of testing more than 19 million PCR test were done). The extensions also made it challenging to align temporary staff employment periods with project activities. All temporary employees were offered industry positions prior to the end of their employment period.

11. Project results

From SACCO, 12 potentially antifungal lactic acid bacteria (LAB) strains (of which 5 were commercialized at the outset) were received and renamed according to current nomenclature. The strains belong to the species *Lacticaseibacillus rhamnosus, Lactiplantibacillus plantarum, Lentilactobacillus parabuchneri* and *Lacticaseibacillus paracasei.* Thirteen molds were collected from yoghurt/skyr, creme fraiche or quark, 9 were *Penicillium* species while 4 were *Mucor* species. Of yeasts, 63 isolates from spoiled acidified dairy products were received.

The yeasts had been identified by donors with different methods and the alignment of two rapid methods employed by the industry with the "gold standard" method was therefore tested. A more recent nanopore-based sequencing technology was furthermore employed to determine if some isolates were identical strains as well as improving the database for this technology.

FTIR identification was made at Arla Foods Innovation Centre in Aarhus and MALDI-TOF MS identification was performed at Arla Foods Nørre Vium. Finally, identification using sequencing of the D1/D2 region of the 26S rRNA gene as well as nanopore-based sequencing was carried out at the University of Copenhagen. The two rapid spectroscopic methods (FTIR and MALDI-TOF MS) applied in industry were very dependent on complete standardization and reliable databases. Compared with the more resource and time consuming 26S rRNA method, the FTIR misidentified 21 out of 63 yeasts with a tendency to identify *Candida parapsilosis* as *Debaromyces hansenii* and *Kluyveromyces lactis* as *Kluyveromyces marxianus.* In one case, a Candida was misidentified as *Candida albicans*, which is an opportunistic pathogen for humans. The MALDI-TOF MS data generally correlated well with sequencing data with few exceptions. However, several strains could not be identified. Two of these isolates, *Candida sojae* and *Kazachstania servazzii,* were not in the database. Three isolates belonging to the species *Clavispora lusitaniae* were also not identified, even though several other isolates from this species were. Furthermore, the two isolates of *Rhodotorula mucilaginosa* could not be identified, which may be due to the red pigment created by this yeast species, since pigmentation has earlier been found to interfere with the ionization process. The three last isolates not identified were *Candida palmioleophila*, *Wickerhamiella pararugosa* (formerly *Candida pararugosa*), and *Trichosporon coremiiforme*. With an expected constant improvement of the database the MALDI-TOF MS is therefore considered a suitable, rapid typing technology for yeasts.

Mold growth on yoghurt at different temperatures was recorded using two technologies. The Ocelloscope allowed detection of growth initiation (cell level) within hours while the Videometer (multispectral imaging) was better suited for estimating growth over a longer period. The *Mucor* strains all grew faster than *Penicillium* strains at all temperatures (5, 16 and 25°C) tested. In a lab media assay (MRS agar with overlay) all the mold strains were strongly inhibited by all 12 LAB strains apart from *P. roquefortii.* Removal of LAB cells diminished the inhibitive effect of the fermentates and exposure to volatiles alone even more so. Several

antifungal metabolites were tested and their minimum inhibitory concentrations determined. It was shown that diacetyl and other compounds induced membrane damages in spores and leakage of intracellular materials (figure 1). The inhibitory effect of diacetyl decreased with increasing temperature and pH.

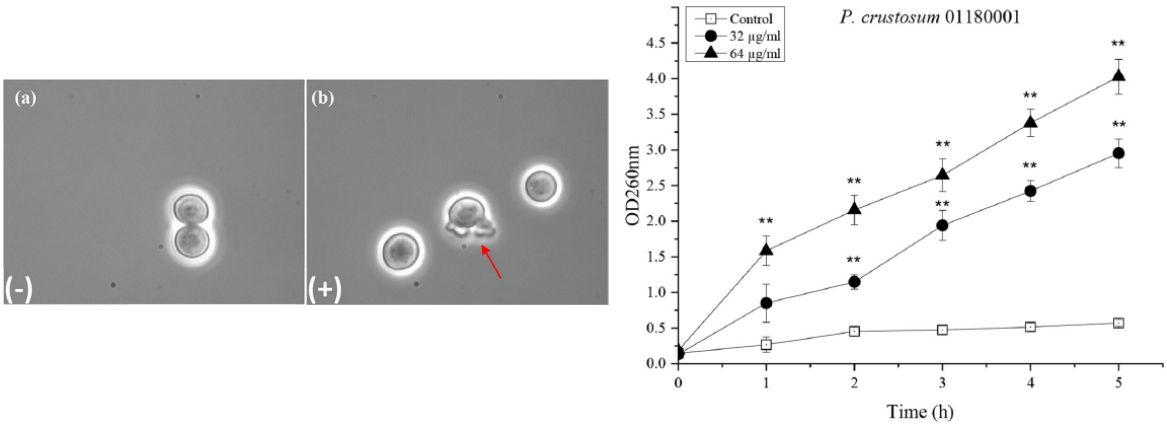

Figure 1. Example of a Penicillium spore exposed to 64 µg/mL diacetyl and the cytoplasmic content leakage detected at OD_{260nm}

When inhibition assays were performed in yoghurt serum as growth media, the LAB strains did not reach same levels and a lower inhibition effect, and a much wider variation was observed both between the bioprotective strains and the various molds. Two tolerant molds (*P. roquefortii* and *Mucor circinelloides*) were tested further to see if mixtures of metabolites would display synergistic actions. Two combinations were found to exhibit synergistic inhibitive action against these molds. Despite the synergistic actions at lower levels of individual compounds, the concentration of these compounds actually recorded in yoghurt did not sufficiently explain the inhibitory effect since their *in situ* concentrations were too low. Test were therefore made to examine the effect of nutrient depletion. Among the essential metals in milk, manganese is found in the lowest amounts (0.03 mg/liter) and LAB are known to contain several manganese transporter genes and accumulate this element as a response to oxidative stress. A range of sensitive molds (5 *Penicillium* and 2 *Mucor* strains) as well as the resistant *P. roquefortii* were tested against 3 individual inhibitory LAB strains and 2 combinations of strains. As expected, the growth of the resistant mold was not affected by manganese addition while manganese restored the growth of all the sensitive strains in a concentration dependent manner up to 0.1 mM (figure 2). In milk-based products, the main inhibitory mechanism therefore seems to be manganese depletion while antifungal metabolites mainly have a supportive role. It should be noted that adding other raw materials to the products beyond milk may change the available manganese amount and thereby the inhibitory effects. It was estimated on the basis of a Danish blueberry-banana yoghurt that the manganese content of this product would be 0.15 mg Mn^{2+}/L, although the amount in this case would not be equally distributed in the matrix.

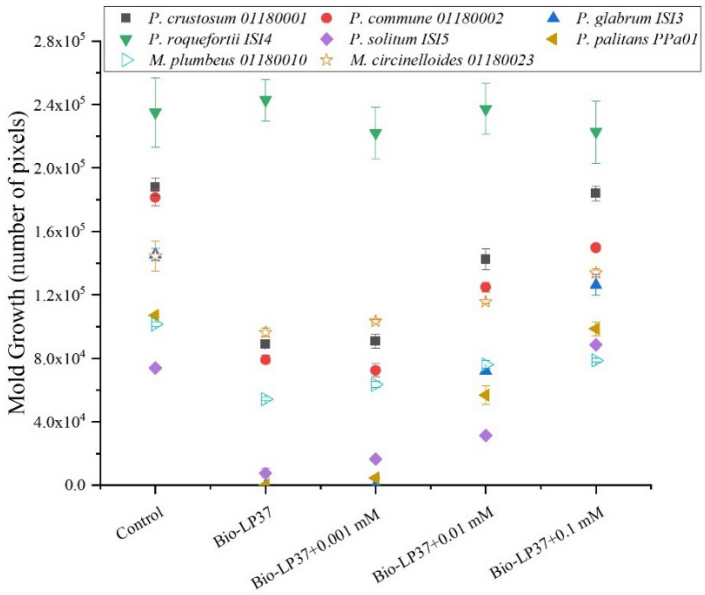

Figure 2. Example of growth inhibition of different molds (except for *P. roquefortii*) in the presence of a bioprotective *L. plantarum* (LP37) in a yoghurt medium and the restoration of growth by the addition of different manganese concentrations (0.001 mM, 0.01 mM and 0.1 mM). Plates were incubated at 25 °C for 5 days. Bars represent the standard error of the mean of three replicates. Bio-LP37 indicated the culture *L. plantarum* LP37.

With regards to the yeasts, all were capable of growing in a yeast specific broth at 5, 16 and 25 °C except one untypeable strain, which did not grow at 5 °C and was therefore excluded. Isolates with similar ON-rep-seq sequencing profiles were also removed. Of the 23 isolates remaining, 8 were not able to grow at 7 °C on yoghurt serum agar. Nine strains produced gas in the yoghurt serum. Fifteen isolates were tested for their sensitivity towards the LAB strains on yoghurt serum agar at two temperatures (7 and 25 °C) on yoghurt serum agar. At 25 °C, there was no inhibition of the yeasts. At 7 °C, 5 of the 15 were not or only very modestly inhibited, one *Pichia kudriavzevii* isolate was sensitive to all LAB strains tested, while the rest showed various degrees of inhibition to some of the LAB strains. Based on the results, 11 yeasts, both sensitive and insensitive, able to grow at 7 °C, were chosen as part of a yeast panel for further studies. Growth test in chemically defined media showed that the yeasts did not have an absolute requirement for manganese under optimal growth conditions. Challenge tests were conducted in yoghurt with and without the LAB strain with the strongest effect, a *L. rhamnosus*, in the initial assays as well as with a combination of this strain and a *L. plantarum.* In the first trial, starting levels of the bioprotective cultures were between 5.3 and 5.95 LOG (CFU/mL) and very little to no effect was observed. A second trial used starting levels between 7.49 and 7.99 LOG (CFU/mL) and followed growth for 39 days. A marked growth delay was seen in 3 out of 11 strains (a slow growing *P. kudravzevii*, a *Rhodotorula mucilaginosa* and one out of two *Torulaspora delbruckii* isolates) while 3 to 4 yeast strain showed modest inhibition. The last strains including a gas producing *Kluyveromyces marxianus* and a fast-growing *Yarrowia lipolytica* showed little to no sensitivity. Addition of manganese had some effect in two of the sensitive strains. A further experiment investigated the effects of low yeast contamination levels (10 CFU/mL) towards high (1000 CFU/mL) in two sensitive and one insensitive strain. A higher contamination level diminished the effect of the bioprotective culture in all cases. In the robust strain (*K. marxianus*) a slight initial inhibition was seen at the low contamination level.

Conclusion: The more efficient bioprotective strains have been identified. The antifungal effect of major metabolic compounds has been investigated as well as the role of nutrient depletion. Molds were generally more susceptible than yeasts to the bioprotective cultures. Some metabolites induce membrane damages, and some combinations exert synergistic effect, but the effect generally requires higher concentrations than found in yoghurt with bioprotective cultures. In the mold cultures examined, the main mechanism of inhibition was shown to be manganese depletion. One mold isolate (*P. roquefortii*) was insensitive to all the cultures. A large collection of spoilage yeasts has been typed with different methods and MALDI-TOF spectroscopy was found to correlate better with sequence data than FTIR spectroscopy although the database still needs improvement for food isolates. The yeasts displayed wide variations in the interactions. Only a minor part of the yeasts were highly sensitive to the bioprotective strains and the sensitivity depended on contamination levels, type and starting levels of the bioprotective cultures as well as temperature. Several common spoilers such as *Yarrowia lipolytica* and *Kluyveromyces marxianus* were among the insensitive strains. A panel of molds and yeasts with different sensitivities towards the tested bioprotective LAB cultures is now available.

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

It is expected that the characterized spoilage yeast and mold panel will be helpful in development and testing of bioprotective cultures in fresh fermented products. The knowledge obtained regarding mechanisms, strain variability, and importance of nutrient availability, growth conditions, contamination levels and more can support the producers of cultures to the industry but also the dairies as such providing a better decision basis when evaluating whether specific microbial shelf life or safety problems can be amended by the use of bioprotective cultures.

13. Communication and knowledge sharing about the project

Papers in international journals (peer reviewed):

1.Shi, C., S. Knøchel. 2020. Susceptibility of dairy associated molds towards microbial metabolites with focus on the response to diacetyl, ***Food Control*** *121*, 107573, https://doi.org/10.1016/j.foodcont.2020.107573

2.Shi, C., S. Knøchel. 2021. Sensitivity of Molds From Spoiled Dairy Products Towards Bioprotective Lactic Acid Bacteria Cultures. ***Frontiers in Microbiology*** (2021) Feb 10;12:631730. doi: 10.3389/fmicb.2021.631730. PMID: 33643260; PMCID: PMC7902714

3. Shi, C., S. Knøchel. 2021. Inhibitory effects of binary combinations of microbial metabolites on the growth of tolerant *Penicillium roqueforti* and *Mucor circinelloides*. ***LWT Food Science and Technology*** 149(6):112039. DOI: 10.1016/j.lwt.2021.112039

4. Shi, C., M. Maktabdar. 2022. Lactic Acid Bacteria as Biopreservation Against Spoilage Molds in Dairy Products – A Review. ***Frontiers in Microbiology*** 12:819684. doi: 10.3389/fmicb.2021.819684.

5. Shi, C., S. Knøchel. 2023. Bioprotection Potential of *Lacticaseibacillus rhamnosus* LRH01 and *Lactiplantibacillus plantarum* LP01 against Spoilage-Associated Penicillium Strains in Yoghurt. ***Molecules*** 28(21):7397. DOI: 10.3390/molecules28217397

A further manuscript on the yeast panel is in preparation.

Easily read papers:

Kanz, L., S. Knøchel, A H. Okholm. Mindre madspild med biobeskyttende kulturer i syrnede mejeriprodukter. **Mælkeritidende 2020**

Susanne Knøchel. Skimmel, gær og biobeskyttende kulturer. **Mælkeritidende 2024.**

Oral presentations at scientific conferences, symposiums etc.: Abstract accepted for Microbial Food and Feed Ingredients conference in 2020. However, due to COVID the conference was postponed to 2021 (after the PhD student had left Europe).

Oral presentations at meetings: Results have been presented at ”Dairy Research Day”.

Other:

Part of the theoretical material has been included in the course ”Dairy microbiology” at University of Copenhagen.

14. Contribution to master and PhD education

Student theses: 1PhD, 2 MSc, 2 BSc theses (one report by two students) and 1 project report (15 ECTS)

PhD Thesis Ce Shi. 2021. Sensitivity of dairy-associated spoilage molds towards lactic acid bacteria and their metabolites.

MSc thesis. Freya Næblerød Jeppe. 2021 Gas Production of Spoilage Yeast and Potentially Bioprotective Lactic Acid Bacteria in Yoghurt Whey

MSc thesis. Maryam Maktabdar. 2021. Effect of manganese on the bioprotective mechanism of lactic acid bacteria with focus on *Lactiplantibacillus plantarum*.

BSc thesis Cathrine Cecilie Petersen & Anne-Sofie Pagh Anthonsen. 2021. Susceptibility of Spoilage Yeasts to Bioprotective Cultures in Dairy Products.

15 ECTS report. Maryam Maktabdar. 2021. Antifungal mechanism of lactic acid bacteria: Suggestions for primers to detect the genes involved in manganese transport in lactic acid bacteria.

15. New contacts/projects

The spoilage potential of selected yeast strains have been investigated further in the project "Improve Dairy Life" (Principal investigator Lene Jespersen, UCPH with participation of Søren Lillevang, Arla Foods).

Selected yeast strains will be tested in a student project with Novonesis (supervisor Nils Arneborg).

A student project involving strains from Chr. Hansen (now Novonesis) has, in collaboration with Cornell University, looked at modelling antifungal interactions as described in the paper:
Nielsen, L., Rolighed, M., Buehler, A., Knøchel, S., Wiedmann, M. & Marvig, C. (2021) Development of predictive models evaluating the spoilage-delaying effect of a bioprotective culture on different yeast species in yogurt. *J. Dairy Sci.* 104:9570–9582 https://doi.org/10.3168/jds.2020-20076

16. References

Aunsbjerg, S.D., A.H. Honoré, J. Marcussen, P. Ebrahimi, F.K. Vogensen, C. Benfeldt, T. Skov, S. Knøchel. 2015a. Contribution of volatiles to the antifungal effect of *Lactobacillus paracasei* in defined medium and yogurt. *International Journal of Food Microbiology* 194: 46–53.

Aunsbjerg, S.D., A.H. Honoré, F.K. Vogensen, S. Knøchel. 2015b. Development of a chemically defined medium for studying foodborne bacterial-fungal interactions. *International Dairy Journal* 45: 48-55.

Axel, C., B. Brosnan, E. Zannini, L.C. Peyer, A. Furey, A. Coffey, E.K. Arendt. 2016. Antifungal activities of three different Lactobacillus species and their production of antifungal carboxylic acids in wheat sourdough. *Applied Microbiology and Biotechnolology* 100(4):1701-1711. doi: 10.1007/s00253-015-7051-x.

Deak, T., 2008. Yeasts in specific types of food, in: Handbook of Food Spoilage Yeasts. CRC press, pp. 117-201.

Delavenne, E., R. Ismail, A. Pawtowski, J. Mounier, G. Barbier, G. Le Blay. 2013. Assessment of lactobacilli strains as yogurt bioprotective cultures. *Food Control*, 30, 206-213.

Fleet, G.H., 1990. Yeasts in dairy products. *Journal of Applied Bacteriology* 68, 199–211.

Frisvad JC, Andersen B, Samson RA (2007b) Association of moulds to foods. In: Dijksterhuis J, Samson RA (eds) Food mycology: a multifaceted approach to fungi and food. Taylor and Francis, Boca Raton, pp 199–239

Honoré, A.H., S. D. Aunsbjerg, P. Ebrahimi, M. Thorsen, C. Benfeldt, S. Knøchel, T. Skov. 2016. Metabolic Footprinting for Investigation of antifungal properties of *Lactobacillus paracasei. Analytical and Bioanalytical Chemistry* 408(1):83-96. doi: 10.1007/s00216-015-9103-6. Epub 2015 Nov 14.

Magnusson, J., K. Ström, S. Roos, J. Sjögren, J. Schnürer. 2003. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS microbiology letters* 219, 129–35.

Pitt, J.I., A. D. Hocking. 2009. The ecology of fungal spoilage, in: Pitt, J.I., Hocking, A.D. (Eds.), Fungi and Food Spoilage. Springer, New York, pp. 3–11.

Siedler, S., M. H. Rau, S. Bidstrup, J.M. Vento, S.D. Aunsbjerg, E.F. Bosma, L.M. McNair, C.L. Beisel, A.R. Neves. 2020. Competitive Exclusion Is a Major Bioprotective Mechanism of Lactobacilli against Fungal Spoilage in Fermented Milk Products. Applied Environmental Microbiology 86:e02312-19.https://doi.org/10.1128/AEM.02312-19

Wang, H., J. Shi, H. Zhang, W. Qi. 2011. A survey of some antifungal properties of lactic acid bacteria isolates from koumiss in China. *International Journal of Dairy Technology*, 64(4), 585–590. doi:10.1111/j.1471-0307.2011.00716.x.

Wolfe, B.E., J.E. Button, M. Santarelli, R.J. Dutton. 2014 Cheese Rind Communities Provide Tractable Systems for *In Situ* and *In Vitro* Studies of Microbial Diversity. *Cell,* 158, 422-433. doi: 10.1016/j.cell.2014.05.041.

Wrent, P., E.-M. Rivas, E. Gil de Prado, J.M. Peinado, M.-I. de Silóniz. 2015. Assessment of the Factors Contributing to the Growth or Spoilage of *Meyerozyma guilliermondii* in Organic Yogurt: Comparison of Methods for Strain Differentiation. *Microorganisms*. 2015;3(3):428-440. doi:10.3390/microorganisms3030428.