Characterizing patatin specific protease activity by high-throughput homo-FRET assay and mass spectrometry

Friis Christensen, Lise; Gregersen Echers, Simon; Overgaard, Michael Toft; Hansen, Egon Bech

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

PROGRAMME & ABSTRACT BOOK

2nd International Conference on
Microbial Food and Feed Ingredients

16-18 November 2021
Copenhagen · Denmark

MiFFI

#MIFFI2021
www.miffi.org

The Danish Microbiological Society
DMS

MiFFI

MIFFI THEMATIC ISSUE 2021

Edited by
Egon Bech Hansen, Dennis Sandris Nielsen, and Gisèle LaPointe

FEMS Microbiology Letters are pleased to present our second Thematic Issue on Microbial Food and Feed Ingredients in collaboration with the MiFFI conferences, a series FEMS is proud to support. The thematic issue features Reviews, Mini-Reviews, and Research Letters and is organised around three major themes:

- Functionality of food cultures
- Pro- and pre-biotics
- Microalgae as feed ingredients.

Read the issue at **academic.oup.com/femsle**

Federation of European Microbiological Societies

OXFORD UNIVERSITY PRESS

Table of contents

Welcome 5

Organisation 6

General information 7

Programme 8

- Tuesday 8
- Wednesday 8
- Thursday 10

Industry symposia 15

Keynote speakers' abstracts 21

Oral abstracts 25

Poster abstracts 47

Poster overview 74

Author index 76

Welcome

Dear participant,

It is a great pleasure to finally welcome you the 2nd International Conference on Microbial Food and Feed Ingredients (MiFFI) 2021 in Copenhagen.

During the two conference days, we can experience a real scientific programme with physical presence of presenters and participants. We can meet with fellow scientists working on food and feed ingredients, we can discuss and argue over posters, and we can even enjoy fermented products together at the end of the day.

The main subjects at the conference are:

- Microorganisms in food and feed
  - Pro- and prebiotics
  - Microbiome
  - Yeast
  - Fungi
  - Whole genome sequences - tool to handle the good guys and the bad guys
- Metabolic models
- Enzymes for food and feed applications
- Microbially derived bioactive compounds
- Applications of cultures, enzymes, and metabolites
- Animal & Human health
- Future trends
- Emerging technologies
- Genetically modified organisms for food applications – the future of the past or still to come
- Regulatory aspects

The topics are addressed from different angles of functionality, safety, and regulatory aspects.

We also have the pleasure to announce that another joint thematic issue on Microbial Food & Feed Ingredients will be published with FEMS Journals (Federation of European Microbiological Societies). We therefore encourage you to submit your manuscripts (full papers and/or reviews), before the 1st of April 2022. The issue will be out during September 2022. We look forward to receiving your papers!

Furthermore, we have arranged some exciting social events, so you will get a chance to network and mingle with colleagues and peers from your field. We hope you will enjoy the conference and your stay in Copenhagen!

On behalf of the Scientific Committee,

Egon Bech Hansen
Chair of the Scientific Committee
Technical University of Denmark, National Food Institute

Organisation

The Scientific Committee

Egon Bech Hansen
Technical University of Denmark,
National Food Institute,
Denmark (Chair)

Dennis Sandris Nielsen
Department of Food Science,
University of Copenhagen, Denmark

Giséle LaPointe
University of Guelph, Canada

Svend Laulund
Chr. Hansen A/S, Denmark

Christel Thea Jørgensen
Bactolife, Denmark

Niels Kjeldsen
SEGES, Denmark

Ildar Nisamedtinov
Lallemand, Estonia

Fergal P. Rattray
University of Copenhagen, Denmark

Karsten Kragh
Iff, Denmark

Jørgen Schlundt
Schlundt Consult, Denmark

Ole Højberg
Aarhus University, Denmark

#MiFFI2021
@MiFFIconference

Use the #MiFFI2021 when posting about the conference!

General information

Conference venue
Marmorhallen
Frederiksberg Campus
University of Copenhagen
Thorvaldsensvej 40
1871 Frederiksberg

Conference language
The conference will be held in English.

Name badges
All participants and exhibitors must wear their name badge in the conference area at all times. The badge must be visible.

Lunch and coffee breaks
Lunch and coffee is available in the exhibition area. See programme for exact time of breaks.

Poster session
The poster area is in the CPSC Foyer.
Please follow the signs.

Social Programme

Welcome reception
(included in the registration fee)

Date	16 November 2021
Time	17.30 - 20.00
Place	CPSC Foyer, Conference venue

Conference dinner
(not included in the registration fee)

Date	17 November 2021
Time	19.00 - 22.30
Place	Copenhagen Food Space
Address	Slagtehusgade 11, 1715 Copenhagen

Speaker information
Please bring your presentation to the session room before your session starts. Your presentation must be uploaded at least 30 minutes before your session starts. A technician will be present to assist in the upload, if necessary. Please bring your presentation on a USB stick.

Unless otherwise agreed all presentations will be deleted after the conference in order to secure that no copyright issues will arise at the end of the conference.

WiFi
Free WiFi is provided throughout the venue by logging on "KU Guest" and creating your own account.

Mobile phones
All mobile phones must be on silent mode during the sessions.

Lost and found
Found items should be returned to the registration desk. If you lose something, please report to this desk for assistance.

Conference secretariat

CAP PARTNER

Nordre Fasanvej 113
2000 Frederiksberg C
Denmark

Tel: +45 70 20 03 05
www.cap-partner.eu

Conference website
www.miffi.org

#MiFFI2021
@MiFFIconference

Use the #MiFFI2021 when posting about the conference!

Programme Tuesday - Wednesday

Tuesday, 16 November

17:00-20:00	**Registration**	
17:30-20:00	**Welcome Reception & Poster Mounting** **CPSC Foyer**	

Wednesday, 17 November

08:30	**MARBLE HALL: REGISTRATION & COFFEE**			
	Room A2-81.01		**Room A2-70.04**	
	PLENARY SESSION I			
	Welcome - Opening ceremony	Chair: **Egon Bech Hansen,** Technical University of Denmark, Denmark		
09.45	Synthetic biology of yeast	**Jens Nielsen**, Chalmers University, Sweden		
10.30	**MARBLE HALL: COFFEE BREAK & EXHIBITION**			
11:00-12:05	**PARALLEL SESSIONS**			
	Room A2-81.01		**Room A2-70.04**	
	Session 1: Pro-prebiotics and the microbiome		**Session 2: Yeast**	
11:00	Introduction	Chair: **Dennis Sandris Nielsen**, University of Copenhagen, Denmark	Introduction	Chair: **John Morrissey,** University College Cork, Ireland
11:05	Selection of specific microbial consortia for future probiotics	**Kaarel Adamberg**, Competence Center of Food and Fermentation Technologies, Taltech, Estonia	Health benefits of foodborne and probiotic yeasts – traits that determine their biological functions	**Lene Jespersen,** University of Copenhagen, Denmark
11:25	The three pillars of probiotics: Documentation, Manufacturing & Application	**Arthur Constantijn Ouwehand**, Iff, Finland	Strain diversity in vitamin B1 yield in *Saccharomyces cerevisiae*	**Rebecca Rocchi,** Wageningen University & Research, The Netherlands
11:45	Mining the gut microbiome of elite athletes for novel probiotics	**Laura Wosinska,** Cork Institute of Technology, Ireland	Can community-based signalling behaviour in *Saccharomyces cerevisiae* be called quorum sensing? A critical review of the literature	**Michela Winters,** University of Melbourne, Australia
11:55	Potential of pectin and its derivatives to improve age-related dysbiosis of the gut microbiota in elderly?	**Fangjie Gu,** University of Copenhagen, Denmark	Identification and succession of spoilage yeasts in the danish feta-type cheese	**Athina Geronikou,** University of Copenhagen, Denmark
12:05	The effect of early probiotic exposure on the preterm infant gut microbiome development	**Yan Hui,** University of Copenhagen, Denmark		
12:05	**MARBLE HALL: LUNCH & EXHIBITION**			
	Room A2-81.01		**Room A2-70.04**	
12:30-13:00	**Industry symposium: Lallemand** Yeast-based nutrients for the optimization of bacteria production			
13:05-13:35	**Industry symposium: Chr. Hansen** Competitive exclusion is a major bioprotective mechanism against spoilage and harmful bacteria in fermented dairy products			
13:35	**CPSC FOYER: POSTER SESSION**			

Wednesday, 17 November

14:00 - 15:05	**PARALLEL SESSIONS**			
	Room A2-81.01		**Room A2-70.04**	
	Session 3: Whole genome sequencing		**Session 4: Metabolic models**	
14:00	Introduction	Chairs: **Jørgen Schlundt,** Denmark & **Frank Møller Aarestrup**, Denmark	Introduction	Chair: **Bas Teusink,** Vrije University, The Netherlands
14:05	Metagenomics for food authenticity, disease, health and surveillance	**Frank Møller Aarestrup,** Aarestrup Lab, Denmark	Modeling approaches for understanding food fermentations	**Bas Teusink,** Vrije University, The Netherlands
14:25	*In silico* prediction of application-relevant phenotypes based on whole-genome sequences	**Kristian Jensen,** Chr. Hansen A/S, Denmark	Revisiting a classic biological phenomenon - The microbial lag phase	**Kevin Verstrepen,** KU Leuven, Belgium
14:45	Decoding the EFSA requirements for microbial safety and the technical challenges	**Adalberto Costessi,** BaseClear BV, The Netherlands	Understanding the influence of fermentation nutrients and (short) peptides is critical for developing effective food cultures and probiotics industrial manufacturing	**Alain M. Sourabié,** Procelys Lesaffre, France
14:55	*Phaeobacter inhibens*, a probiotic to control bacterial infections in aquaculture	**Eva Sonnenschein,** Technical University of Denmark, Denmark	Constraint-based metabolic modelling in microbial food biotechnology: *Streptococcus thermophilus* as a case study	**Martin Holm Rau,** Chr. Hansen A/S, Denmark
15:05	**MARBLE HALL: COFFEE BREAK & EXHIBITION**			
	Room A2-81.01		**Room A2-70.04**	
	PLENARY SESSION II			
	Introduction	Chair: **Dennis Sandris Nielsen**, University of Copenhagen, Denmark		
15:30	Key lecture: Domestication of yeast in nature and for biotechnology	**John Morrissey**, University College Cork, Ireland		
16:10	Key lecture: Fermentation of plant proteins: Influence on flavor, texture and bio-preservation	**Herwig Bachmann,** NIZO, The Netherlands		
16:45	**BREAK**			
19:00	**CONFERENCE DINNER (Copenhagen food space)** NB: Conference Dinner ticket must be purchased separately.			

Programme Thursday

Thursday, 18 November

	Room A2-81.01		Room A2-70.04	
	PLENARY SESSION III			
9:00-10.00	**Future trends and regulatory affairs: Challenges and opportunities**			
09:05	Getting back to the roots of Fermentation: Preserving Food and Extending Shelf Life	**Francois Bourdichon,** UIDF – SCMH, France		
09:25	Food conservatisms: EU regulatory status assessment of food micro-organisms	**Bernd van der Meulen,** European Institute for Food Law, The Netherlands		
09:45	**Discussion**			
10:00	**MARBLE HALL: COFFEE BREAK & EXHIBITION**			
10:30-11:35	**PARALLEL SESSIONS**			
	Room A2-81.01		**Room A2-70.04**	
	Session 5: Enzymes for food and feed applications		**Session 6: Microbially derived bioactive compounds**	
10:30	Introduction	Chair: **Karsten Kragh,** Iff, Denmark	Introduction	Chair: **Fergal Rattray,** University of Copenhagen, Denmark
10:35	Enzyme application in food	**Jens Frisbæk Sørensen,** Iff Denmark	The biotic family - the possibilities for bioactive microbiota modulation components	**Seppo Salminen**, University of Turku, Finland
10:55	Enzyme application in feed	**Albert van Dijk,** Schothorst Feed Research, The Netherlands	Engineering yeasts for feed and food ingredients	**Irina Borodina,** The Novo Nordisk Foundation Center for Biosustainability, Denmark
11:15	Substrate specificity of Extracellular serine proteinases from lactic acid bacteria	**Egon Bech Hansen,** Technical University of Denmark, Denmark	Microbial proteins: Moving from feed to food applications aided by proteomics and bioinformatics	**Simon Gregersen,** Aalborg University, Denmark
11:25-11:35	Characterizing patatin specific protease activity by high-throughput homo-FRET assay and mass spectrometry	**Lise Friis Christensen,** Technical University of Denmark, Denmark	Postbiotics impact microbiota, host behaviour and colitis - an example using heat-treated lactobacilli	**Alicja Warda,** APC Microbiome, Ireland
	Room A2-81.01		**Room A2-70.04**	
11:40-12:10	**Industry Symposium: DSM** Enzymes as a solution for the challenges in plant based dairy alternatives			
12:10	**MARBLE HALL: LUNCH & EXHIBITION**			
13:10	**CPSC FOYER: POSTER SESSION**			

Thursday, 18 November

14:00-14.55	PARALLEL SESSIONS			
	Room A2-81.01		Room A2-70.04	
	Session 7: Microbiome/Animal & Human Health		**Session 8: Enrichment of plant-based foods via fermentation**	
14:00	Introduction	Chair: **Filip Van Immerseel,** U Gent, Belgium	Introduction	Chair: **Dennis Sandris Nielsen**, University of Copenhagen, Denmark
14:05	Steering of the intestinal microbiota composition in production animals, to reduce *Salmonella* in humans	**Filip Van Immerseel,** U Gent, Belgium	Enrichment of plant based foods by fungi	**Han Wösten**, Utrecht University, The Netherlands
14:25	Investigation and manipulation of gut bacterial functions – the example of glycerol/diol dehydratase activity	**Clarissa Schwab,** Aarhus University, Denmark	Metabolic response of lactic acid bacteria strains during brewers' spent grain fermentation (10 min)	**Marta Acin Albiac,** Free University of Bozen Bolzano, Italy
			Brewers' spent grain as substrate for synthesis of dextran by lactic acid bacteria: regulation of dextransucrases and fermentation performance (10 min)	**Prabin Koirala,** University of Helsinki, Finland
14:45	Evaluation of humic acids as functional feed additive on performance, metabolic parameters and gut microbiota of weaned piglets	**Mattoe Dell'Anno,** Università degli Studi di Milano, Italy	Lactic acid bacteria fermentation as a tool to improve the antioxidant properties of brewers' spent grain: bioprocess set-up, characterization and application in pasta making	**Michela Verni,** University of Bari Aldo Moro, Italy
14:55	High Throughput in vitro characterization of Pectins for Pig(let) Nutrition and Health	**Maria Wiese,** TNO, The Netherlands	Evidencing fermented rapeseed meal using lactic acid bacteria is an alternative for discarding high dose of zinc oxide in piglet production	**Ninfa Rangel Pedersen,** Fermentation Experts, Denmark
15:05	**QUICK BREAK – GO TO ROOM A2-81.01**			
15:10-15:30	**Room A2-81.01: CLOSING SESSION CLOSING REMARKS AND POSTER PRIZES**			

PLENARY SESSION III & PANEL DISCUSSION

Future trends and regulatory affairs: Challenges and opportunities

Chair Svend Laulund, Chr. Hansen, Denmark
Day Thursday, 18 November 2021
Time 09.00-10.00

Today's food industry is looking for means of producing safe food products with adequate shelf life, thus reducing food waste and meeting the consumer demands for reduced use of preservatives. Fermentation is the oldest food preservation technique going back to Neolithic times. While the traditional use of cultures in fermented foods refers to their positive action on product properties (texture, aroma, digestibility, nutritional values, aspect ...), and at the same time inhibit spoilage microflora and improve food safety. But this protective effect can also be use with minimum texture or aroma properties and solely enhanced food safety and extended shelf life of foods by indigenous and/or intentionally added microflora. It adds on an extra hurdle for perishable food products.

Food cultures used directly in food production are regarded as food ingredients in most of the World. The "rediscovery" for its role of preserving food, meets a hurdle as food cultures can wrongly be considered as "new" food additives by regulatory agencies.
The legal status of food cultures as a food ingredient in the EU, along with data required to substantiate their safety, has been addressed in several publications, but debates are ongoing on criteria to be meet by those ingredients, especially when used in new recipes, to be considered as "food" vs. having to be classified as a "food additive" or as a "novel food".

In this context, a multidisciplinary group of professionals, bringing expertise in science, law and food processing has developed an in-depth analysis of the European legal acts governing the use and labelling of foods, a guidance to research institutes & the industry for determining the conditions under which a certain food culture recipe/combination shall be considered as a "food ingredient".

Speakers

Francois Bourdichon
UIDF – SCMH, France

Bernd van der Meulen
European Institute for Food Law, The Netherlands

The session will turn into a panel discussion.

FNI

FERMENTATION NUTRIENTS BY LALLEMAND

Our range of products and technical expertise are combined to support the fast-growing demand in industrial microbiology-based industries.

We develop, produce and supply yeast-based solutions to optimize the industrial production and performance of a broad range of organisms (bacteria, yeasts, moulds, fungi and others).

Fermentation Nutrients key application domains

MICROBIAL BIOMASS PROBIOTICS CULTURES

Starter cultures | Lactic acid bacteria
Probiotic strains | Plant care microbes

Increased biomass yield
Improved stability and performance of final products

BIOACTIVE MOLECULES AND FINE CHEMICALS

Enzymes | Amino acids | Vitamins
Antibiotics | Flavors and fragrances

Higher titer in metabolites production
Better stability and performance
Easier downsteam processing

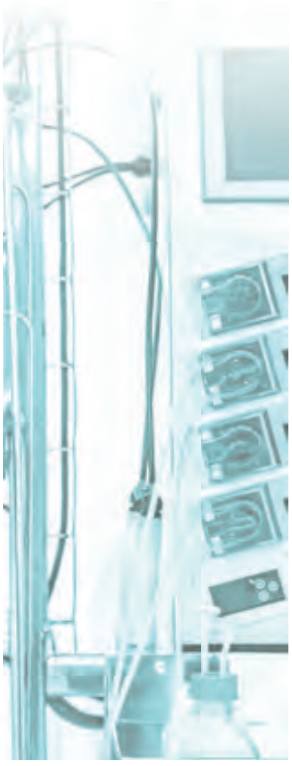
CULTURE MEDIA AND DIAGNOSTIC

Ready-to-use media | Culture media
Production of vaccines | Recombinant protein

Ultra clear filterable products
Better growth performance in petri dishes

OUR RANGE OF PRODUCTS

	Probiotics		Recombinant proteins						
	Lactobacillus	*Bifidobacteria*	*E. coli*	*S. cerevisiae*	*Bacillus*	*Enzymes antibiotics*	*Plant care*	*Media*	*Organic*
FNI 100	●	●	●	●	●	●	●	●	
FNI 103					●		●		
FNI 105	●	●	●	●		●		●	
FNI 110								●	
FNI 125	●	●	●	●		●	●		
FNI 150	●	●	●	●		●	●		
FNI 300					●	●	●		
FNI 307					●	●	●		
FNI 320	●	●			●	●	●		
FNI 327					●	●			
FNI 602	●	●							
FNI 800	●	●	●	●					
FNI 850	●	●	●	●					
FNI 837 Bio									●



Lallemand Fermentation Ingredients

LALLEMAND

Industry Symposium

LALLEMAND BIO-INGREDIENTS

Wednesday, 12.30-13.00
Room A2-81.01

YEAST-BASED NUTRIENTS FOR THE OPTIMIZATION OF BACTERIA PRODUCTION

Speaker

David Guerrand
PhD, Biotech Business Director, Lallemand Bio-Ingredients

Description

Lallemand is a privately owned Canadian company, a leader in the development, production and marketing of yeast, bacteria and specialty ingredients. Lallemand Bio-Ingredients produces inactive yeast and yeast derivatives for the savory, health and fermentation markets.

The presentation will mainly focus on the application of yeast-based nutrients to produce probiotics. Examples will be brought highlighting the importance of specific nutrients to optimize the yield and stability of bacteria when produced at industrial scale.

Want to learn more about how our fermentation-enabled bioprotection works, scan the QR code

CHR. HANSEN

Improving food & health

Industry Symposium

Wednesday, 13.05-13.35
Room A2-81.01

COMPETITIVE EXCLUSION IS A MAJOR BIOPROTECTIVE MECHANISM AGAINST SPOILAGE AND HARMFUL BACTERIA IN FERMENTED DAIRY PRODUCTS

Speakers

Solvej Siedler
Ph.D, Principal Scientist, Chr. Hansen A/S

Stina Dissing Aunsbjerg Hoff
Ph.D, Senior Application Scientist, Chr. Hansen A/S

Description

Conscious consumers demand natural solutions to keep their food healthy and fresh during storage, simultaneously reducing food waste. The use of "good bacteria" to protect food against spoilage and harmful organisms has a long, successful history, even though the molecular mechanisms are not always fully understood. In this study, we show that the depletion of free manganese can be a major bioprotective mechanism of lactobacilli in dairy products. Hence, through the natural mechanism of nutrient depletion, the use of dedicated bioprotective lactobacilli constitutes an attractive route to extend shelf life and increase safety in fermented dairy products.

A prominent feature of lactic acid bacteria (LAB) is their ability to inhibit growth of spoilage and harmful organisms in food, but hitherto research efforts to establish the mechanisms underlying bioactivity focused on the production of antimicrobial compounds by LAB. We show that competitive exclusion, i.e., competition for a limited resource by different organisms, is a major mechanism of growth inhibition by lactobacilli in fermented dairy products. The depletion of the essential trace element manganese by two Lactobacillus species was uncovered as the main mechanism for growth inhibition of dairy spoilage yeast and molds. A manganese transporter (MntH1), representing one of the highest expressed gene products in both lactobacilli, facilitates this exhaustive manganese scavenging. Expression of the mntH1 gene was found to be strain dependent, affected by species coculturing and the growth phase. Further, deletion of the mntH1 gene in one of the strains resulted in a loss of bioactivity, proving this gene to be important for manganese depletion. Manganese scavenging emerges as a common trait within the Lactobacillus genus, but differences in expression result in some strains showing more bioprotective effect than others. In summary, competitive exclusion through ion depletion is reported as a novel mechanism in LAB to delay the growth of spoilage and harmful contaminants in dairy products.

DSM, a world leader in biotechnology

At Royal DSM, we see biotechnology as an important building block to address climate change, resource scarcity, and circularity, and to optimize the global food system. Building on 150 years of expertise, today DSM is a global purpose-led leader in biotechnology, developing a broad range of sustainable, bio-based products and solutions for better health through nutrition.

DSM's biotechnology competences offer world-class science and innovation, organized in a global network of R&D and manufacturing facilities in Europe, China, Brazil, and the USA. Here, collaborating with our partners from governments, business, and academia, we work to expand the possibilities of biotechnology and maximize its potential to provide solutions to help sustain and restore healthy populations, ecosystems, and societies – contributing to a world we all want to live in: healthy people, healthy animals, and a healthy planet.

www.dsm.com

NUTRITION · HEALTH · SUSTAINABLE LIVING

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.

Industry Symposium

Thursday, 11.40-12.10
Room A2-81.01

ENZYMES AS A SOLUTION FOR THE CHALLENGES IN PLANT BASED DAIRY ALTERNATIVES

Speakers

MSc PhD Anne José Klok
Innovation Manager Milk and Plant-Based Dairy Alternatives DSM

Description

Consumer motivations are more than ever based on health and sustainability. As a result, the plant based dairy alternatives market is growing rapidly and there is a great willingness to try new products. This huge opportunity drives both new players to venture out in plant-based dairy, as well as existing players to be innovative and diversify.

The main challenge for manufacturers is to develop well tasting products with appealing texture and good nutritional value, based on a much wider range of substrates than those available for traditional dairy products. Enzymes are a key tool to address these issues, as they can modify food properties and as such improve taste, texture, and health of plant-based dairy alternatives.

Oat drinks are outpacing the plant-based drinks market and enzymes play a central role in production of these beverages. For example, the right choice of amylases will determine mouthfeel and sweetness, beta-glucanases and proteases can be used to reduce viscosity and phytase can be used to liberate minerals such as calcium, by breaking down phytic acid. These examples clearly illustrate that enzymes are key tools for creating appealing plant based dairy alternatives. Yet to tackle all challenges, there is a need for integrated solutions where the power of enzymes is combined with innovative cultures, natural stabilizers and fortification with plant proteins and vitamins.

Keynote speakers' abstracts

[K1] SYNTHETIC BIOLOGY OF YEAST

Jens Nielsen[1,2,3]

[1] BioInnovation Institute, Denmark
[2] Department of Biology and Biological Engineering, Chalmers University of Technology, Sweden
[3] Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Denmark

Synthetic Biology relies on the Design-Build-Test cycle. This cycle includes technologies like mathematical modeling of metabolism, genome editing and advanced tools for phenotypic characterization. In recent years there have been advances in several of these technologies, which has enabled faster development of metabolically engineered strains that can be used for production of fuels and chemicals.

The yeast *Saccharomyces cerevisiae* is widely used for production of foods and beverages, but also food ingredients and dietary supplements. Through metabolic engineering of this yeast a number of novel industrial processes have been developed over the last 10 years. Besides its wide industrial use, *S. cerevisiae* also serves as an eukaryal model organism, and many systems biology tools have therefore been developed for this organism. These tools can be used for detailed phenotypic characterization as well as for metabolic design.

In this lecture it will be demonstrated how the Design-Build-Test cycle has allowed for development of yeast cell factories for production of a range of different fuels and chemicals. Some examples of different technologies will be presented together with examples of metabolic designs, in particular for development of platform strains that can be used for production of a fatty acid derived products, e.g. fatty alcohols. It will be argued that with advancement in genome-editing technologies and novel methods for rapid phenotypic screening, advancement in the field is hampered by our design abilities, i.e. to predict genotype-phenotype connections. For this genome-scale metabolic models is a strong technology, and in the presentation recent advancements in the integration of mathematical modeling with multi-omics analysis for cell factory design will be presented.

[K2] DOMESTICATION OF YEAST IN NATURE AND FOR BIOTECHNOLOGY

John Morrissey[1]

[1] University College Cork, Ireland

Yeast are single-celled microbial eukaryotes that display tremendous diversity in terms of habitats, genomes and biology. The majority of species probably remain to be described. but, there are some well-studied species that are very important to humans since they are responsible for the production of many fermented foods and beverages. These products include alcoholic beverages, fermented meats, dairy products and essential daily nutrients like coffee and chocolate! Some of these fermented foods have been produced by traditional process for thousands of years and particular yeast species and strains have been inadvertently, but beneficially, domesticated by humans. These domesticated strains are often the basis of starter cultures used for commercial production. Currently, we use only a small proportion of the potential diversity that is available and one of the big opportunities in modern biotechnology is to find ways of taking advantage of the diversity nature offers in order to create new products that appeal to consumers or to improve production processes. One challenge is to understand genotypic and phenotypic diversity and this is being strongly enabled by improved DNA sequencing methods and molecular technology that enables us to work with new isolates and species. The development of CRISPR-based genome engineering and other synthetic biology tools offer the potential to engineer yeasts to produce metabolites of interest. This presentation will focus on *Kluyveromyces* yeasts and explain how fundamental population studies have yielded insight with applications for biotechnology. It will also discuss how modern tools have allowed us engineer *Kluyveromyces* as a chassis for the production of aromatic compounds with flavour activity.

[K3] FERMENTATION OF PLANT PROTEINS: INFLUENCE ON FLAVOR, TEXTURE AND BIO-PRESERVATION

Herwig Bachmann[1], Renske Janssen[1], Marjon Wells-Bennik[1], Wim Engels[1]

[1] *Nizo, Ede, Netherlands*

The replacement of animal derived proteins with plant proteins is an essential step to ensure a globally sustainable and healthy food supply. This transition has been gaining momentum over recent years with an increasing demand for functional plant proteins. While the majority of products aim to replace either dairy or meat there are still numerous technological challenges to achieve similar product properties. These challenges include nutritional parity and the optimization of flavor and texture. However, many plant-based ingredients contain viable, spore forming organisms that impact on food safety and product shelf-life and which need to be controlled. One approach to improve functional properties of plant protein is by fermentation. For this we make use of the biodiversity available in food grade culture collections and screen them for desired properties such as fast acidification, flavor formation and off-flavor removal. Microbial genome information and experimental data of the strains is used to predict strain properties through machine learning. Besides harnessing biodiversity, we also use experimental evolution to adapt microbial strains with desired properties to plant protein-based products and ingredients. This allows to identify cultures which show fast product acidification and thereby increases their bioprotective properties. Other examples for adding functionality with fermentation to plant proteins include positive alterations of volatile profiles, texture and the prevention of fungal outgrowth in plant based products. Together this allows us to tailor fermentations to improve the quality of plant protein ingredients and products.

Where science
& creativity meet

BIOSCIENCE

FOR LIFE

Health & Biosciences

At IFF, we're captivated by bioscience - fascinated by its potential, inspired by its vitality. We channel that passion into sustainable, life-enhancing technologies across a broad number of industries. By creatively applying our extensive expertise to customer and consumers' needs, we harness the science that breathes life into us, and infuses life into the world.

iff-health@iff.com
iff.com
howaru.com

Oral abstracts

Oral abstracts

SESSION: PRO-PREBIOTICS AND THE MICROBIOME

[O01] SPECIFIC SELECTION OF MICROBIAL CONSORTIA BY CONTINUOUS CULTIVATION FOR FUTURE PROBIOTICS

Kaarel Adamberg[1,2], Grete Raba[1], Signe Adamberg[1]

[1]*Tallinn University of Technology, Tallinn, Estonia*
[2]*Center of Food and Fermentation Technologies, Tallinn, Estonia*

Colon microbiota, composed of hundreds of different species, is closely associated with our health. Systematic evaluation of the underlying mechanisms of the complex microbial interactions can be done by combining *in vitro* cultivation methods and up-to-date analytics. A novel approach of microcalorimetry and change-stat culture for selective enrichment of fecal microbial consortia to develop or reproduce symbiotic functional mixed cultures is proposed. Cultivation methods called A-stat or D-stat were used, in which the studied parameter is changed in a rate that allows simultaneous adaptation of bacteria.

The effect of acidity (pH 5.9-7.8) was investigated at two dilution rates (D_{low} = 0.05 and D_{fast} = 0.2 1/h), corresponding to "fast" and "slow" colonic transit rates. Defined base medium, supplemented by a single dietary fibre and porcine mucin, was used. Responses of fecal microbiota were analyzed using 16S-rDNA sequencing and metabolic patterns.

Small scale batch experiments in microcalorimeter indicated that propionate-producing bacteria became enriched on xylan and arabinogalactan while butyrate-producing bacteria on levan and pectin. The A-stat and De-stat experiments on pectin and xylan showed that the abundances of most bacterial taxa were controlled by the dilution rate. *Bacteroides ovatus*, *Bacteroides vulgatus* and *Faecalibacterium* were prevalent within the whole range of dilution rates from 0.05 to 0.2 1/h. *Akkermansia muciniphila* and Ruminococcaceae UCG-013 were significantly enriched at D_{low} compared to D_{high}, whilst *Bacteroides caccae*, Lachnospiraceae unclassified and *Escherichia coli* clearly preferred D_{high}. Respectively, the molar ratio of acetate, propionate and butyrate was 5:2:1 at D_{low} and 14:2:1 at D_{high}. Reproducible adaptation of the fecal microbiota was shown in changestat - the continuous culture with a changing dilution rate. Specific growth rate was found to have a more pronounced effect on the microbial composition and function compared to that of pH.

Information on reproducing fecal microbial consortia can be used for various clinical and biotechnological applications, for example for propagation of multi-strain probiotics or mixtures for fecal transplantation.

[O02] THE THREE PILLARS OF PROBIOTICS: DOCUMENTATION, MANUFACTURING & APPLICATION.

Arthur Ouwehand[1]

[1]*International Flavors and Fragrances, Kantvik, Finland*

The successful development of probiotic foods and dietary supplements rests on three pillars; each with their specific challenges and opportunities. First, strain production; this depends on selecting the right strain with promising technological properties and safety profile. Further the manufacturing of the strain in a stable format at sufficiently high yield, following regulatory and customer requirements on culture media ingredients and other processing aids. The second pillar consists of the preclinical and clinical studies to document that the strain is a probiotic and exerts a health benefit on the host, the consumer. Especially when aiming for a regulator approved health claim, clinical studies need to be thoroughly performed; following appropriate ethical, scientific and regulatory guidelines. Finally, the probiotic will need to be incorporated in a product that can be brought to the consumer; a dietary supplement or a functional food. Other, non-food delivery formats are, of course, also possible but will not be discussed here. Because of the live nature of probiotics, specific challenges may need to be dealt with. Although experience from other strains is helpful in the process, the development is strain specific. Commercialisation and marketing of probiotics are strictly but differently regulated in most jurisdictions; defining what can and cannot be stated.

SESSION: PRO-PREBIOTICS AND THE MICROBIOME

[003] MINING THE GUT MICROBIOME OF ELITE ATHLETES FOR NOVEL PROBIOTICS

Laura Wosinska[1], Paul D. Cotter[2], Orla O`Sullivan[2], Caitriona Guinane[1]

[1] *Cork Institute of Technology, Cork, Ireland*
[2] *Teagasc Moorepark, United Kingdom*

Exercise confers numerous physiological effects on the host, from mood regulation to improved cardiovascular symptoms and alleviation of fatigue and anti-inflammatory effects. There is also accumulating evidence that physical fitness influences the gut microbiome and as a result, health. Exercise has been shown to alter the gut microbial community by increasing and/or decreasing many bacterial species. Therefore, it could be postulated that, exercise-induced alterations in the gut microbiome could have consequences on health parameters crucial to athletic performance, specifically, immune function, lower susceptibility to infection and inflammatory response.

The interest in probiotics and their potential use in sports has received a lot of attention in recent years, particularly focusing on attenuating symptoms of overtraining. A condition of overtraining can occur if the recovery period is not balanced with the training load. Overtraining is a huge concern in athletes and can put the athlete at a risk of developing infections, asthma, gastrointestinal complaints as well as depression and anxiety. Symptoms such as immunity suppression and chronic fatigue are also common. Certain probiotic strains have the potential to produce antimicrobial peptides, have anti-inflammatory activity or modulate tight-junction proteins and therefore may offer a strategy to prevent or alleviate the symptoms of overtraining and allow for continued success without the drawback of the aforementioned symptoms.

The current state of knowledge allows us to mine through elite athlete faecal samples and screen for novel probiotics. The novel probiotic(s) will have the potential to be used in athletes and the general population. This can be achieved through the more traditional culture-based screening methods and the more refined next generation sequencing technologies.

[004] POTENTIAL OF PECTIN AND ITS DERIVATIVES TO IMPROVE AGE-RELATED DYSBIOSIS OF THE GUT MICROBIOTA IN ELDERLY

Fangjie Gu[1], Nadja Larsen[2], Nélida Pascale[1], Beinta Marr[1], Sune Allan Petersen[1], Frederique Respondek[1], Birgit Michelsen[3], Lene Jespersen[2]

[1] *Cp Kelco, Lille Skensved, Denmark*
[2] *University of Copenhagen, Frederiksberg, Denmark*
[3] *Deerland Probiotics & Enzymes*

The elderly population is rapidly increasing nowadays and is projected to reach 1.4 billion globally by 2030. Elderly individuals are characterized by age-associated dysbiosis of the gut microbiotaand immunosenescence. Pectins are considered emerging prebiotics, utilization of which by the gut microbiota supports the growth of primary pectin-degraders within genera *Bacteroides* and *Prevotella*. However, the effect of pectins on the various other gut commensals is not clear. The aim is to investigate the ability of pectic substrates to modulate the human gut microbiota regarding relative abundances of the main bacterial populations, microbial diversity and accumulative production of short chain fatty acids (SCFA) using *in vitro* fermentations. Three pectic substrates were selected for the present study, including one pectin polysaccharide of citrus origin, one modified pectin and one oligosaccharide, with the latter two being prepared from the first one. One inulin sample was employed as comparison to the pectins. Faecal inoculums were prepared from individual faecal samples that were collected from five healthy elderly volunteers (70 – 75 y.o.) and from five younger ones (30 – 35 y.o.), by dilution with phosphate buffered saline. *In vitro* microbiota fermentations were performed using an in-house MiniGut colon model, which is a batch fermentation model featuring monitored and controlled temperature and pH. Samples were withdrawn before and after 24-hour fermentation for measurement of microbiota composition and SCFA profiles. Ongoing experiments include 16S rRNA gene sequencing by GridION nanopore (Oxford Nanopore Technologies) and SCFA determination by gas chromatography- mass spectrometry. The future results will provide new knowledge of the potential of pectin and pectin-based synbiotics to counteract age-related imbalances in humans, which can facilitate selection and development of new products targeting the elderly population.

Oral abstracts

SESSION: PRO-PREBIOTICS AND THE MICROBIOME

[O05] THE EFFECT OF EARLY PROBIOTIC EXPOSURE ON THE PRETERM INFANT GUT MICROBIOME DEVELOPMENT

Yan Hui[1], Birgitte Smith[2], Martin Steen Mortensen[1], Lukasz Krych[3], Søren Johannes Sørensen[1], Gorm Greisen[4], Karen Angeliki Krogfelt[5], Dennis Sandris Nielsen[3]

[1] *University of Copenhagen, Copenhagen, Denmark*
[2] *Hvidovre Hospital, Denmark*
[3] *University of Copenhagen, Frederiksberg, Denmark*
[4] *Rigshospitalet, Copenhagen University Hospital, Denmark*
[5] *Statens Serum Institut, Copenhagen, Denmark*

Premature birth, especially before 32 weeks of gestation, is associated with increased risk of neonatal morbidity and mortality. Prophylactic use of probiotics has been suggested to protect preterm infants via supporting a healthy gut microbiota (GM) development, but heterogenous conclusions exist between different strains and doses.

In this study, we profiled the GM of 5, 10 and 30-day fecal samples from two cohorts of preterm neonates (born < 30 weeks of gestation) recruited in one Danish neonatal intensive care unit. One cohort (*n* = 165) was recruited from September 2006 to January 2009 before probiotics were introduced in the clinic. The second cohort (*n* = 87) was recruited from May 2010 to October 2011 after adopting the supplementation policy of *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium animalis* ssp. *lactis* BB-12. Through V3-V4 region 16S rRNA gene amplicon sequencing, a distinct increase of *L. rhamnosus* and *B. animalis* was found in the fecal samples of neonates supplemented with probiotics. The probiotic supplementation was found to be associated with distinct reduction of *Weissella*, *Veillonella* spp. and the opportunistic pathogen *Klebsiella*. However, regardless of the probiotic use, the preterm GM went through similarly patterned progression of certain bacterial populations in the first 30 days of life. The initially predominant *Staphylococcus* and *Weissella* were overtaken by *Veillonella*, *Enterococcus* and *Enterobacteriaceae*, while potential nosocomial pathogens *Citrobacter* and *Chryseobacterium* species gradually phased out. These longitudinal changes were probably related to the increasing ratio of oral feeding with age, where the nurtured bacteria competitively excluded the early inhabitants from the hospital environment.

In conclusion, probiotic supplementation to preterm neonates affected the early gut bacterial colonization, but did not change the longitudinal progression of certain bacteria in the neonatal period.

SESSION: YEAST

[O06] HEALTH BENEFITS OF FOODBORNE AND PROBIOTIC YEASTS – TRAITS THAT DETERMINE THEIR BIOLOGICAL FUNCTIONS

Lene Jespersen[1]

[1] *University of Copenhagen, Department of Food Science, Denmark*

Yeasts do not only play a significant role in the technological production of many fermented food and beverages. Both industrial yeasts as e.g. *Saccharomyces cerevisiae* and non-conventional yeasts might additionally offer nutritional improvements of the products. It is generally known that different yeast species play significant roles in the degradation of anti-nutritional factors as e.g. phytic acid, production of vitamins and delivery of other micronutrients. However, food-associated yeasts and yeasts administered as dietary supplements may additionally offer other health beneficial effects. Yeasts have been found to interact with the host immune system, degrade bacterial toxins and enhance the trans-epithelial barrier. Specifically *"Saccharomyces boulardii"* (taxonomically belonging to *S. cerevisiae*) is used for treatment of acute infectious diarrhoea, including *Clostridium difficile* infections. Probiotic yeasts are also used in the treatment of inflammatory bowel diseases due to their ability to modulate human immune responses.

Though it is currently accepted that our gastrointestinal tract contains an overwhelming number of microorganisms having increasingly recognized impact on human health, our knowledge on how yeasts influence human health in a positive manner is currently rather scattered. The aim of the presentation is therefore to give an overview on existing knowledge within the area and to present a number of scientific results dealing specifically with the effect of yeasts on improvement of nutritional quality of fermented foods, their interactions with the human immune system and their potential role as future probiotics.

Key words: probiotic yeasts, anti-nutritional factors, immunological responses, *Saccharomyces boulardii*

SESSION: YEAST

[007] STRAIN DIVERSITY IN VITAMIN B1 YIELD IN *SACCHAROMYCES CEREVISIAE*

Rebecca Rocchi[1], Eddy J. Smid[1]

[1] *Wageningen University, Wageningen, Netherlands*

Yeasts are highly exploited for the production of flavours. Yeast biomass itself is valuable in food applications by being converted to yeast extracts, which are used for their umami flavour as an alternative to glutamate[1]. Yeasts can also produce numerous vitamins. *S. cerevisiae* produces thiamine (vitamin B1). As awareness over health is gaining more attention, it would be interesting to increase thiamine levels in yeast extracts to use as flavour in (fermented) meat analogues, among other possible substrates. In this study the capability of different strains of *S. cerevisiae* to produce vitamin B1 is investigated. A screening of different yeast strains, isolated from various food sources, was carried out in Wickerham minimal medium lacking amino acids and thiamine. In this screening intracellular and extracellular thiamine production was measured among the different strains. The screening showed large variation in thiamine production and excretion among the tested strains, ranging in the ppm levels for intracellular thiamine content and ppb for extracellular. Intracellular thiamine levels are shown to be growth stage dependent.

1) Aygul Alim and others, 'The Behavior of Umami Components in Thermally Treated Yeast Extract', Food Research International, 120.August 2018 (2019), 534–43 <https://doi.org/10.1016/j.foodres.2018.11.002>.

SESSION: YEAST

[008] CAN COMMUNITY BASED SIGNALING BEHAVIOUR IN *SACCHAROMYCES CEREVISIAE* BE CALLED QUORUM SENSING? A CRITICAL REVIEW OF THE LITERATURE

Michela Winters[1], Nils Arneborg[2], Kate Howell[1], Rudi Appels[1]

[1] *University of Melbourne, Parkville, Australia*
[2] *University of Copenhagen*

Quorum sensing is a well-described mechanism of intercellular signalling among bacteria that involves cell-density dependent chemical signal molecules. The concentration of these quorum sensing molecules increases proportional to cell density until a threshold value is exceeded which triggers a community-wide response. In this review we propose that intercellular signalling mechanisms can be associated with a corresponding ecological interaction type based on similarities between how the interaction affects the signal receiver and producer. Thus, we do not confine quorum sensing, a specific form of intercellular signalling, to only cooperative behaviours. Instead we define it as cell-density dependent responses which occur at a critical concentration of signal molecules and through a specific signalling pathway. For fungal species, the medically important yeast *Candida albicans* has a well-described quorum sensing system while this system is not well described in *Saccharomyces cerevisiae,* which is involved in food and beverage fermentations. The more precise definition for quorum sensing proposed in this review is used to consider the studies suggesting that *S. cerevisiae* may undergo intercellular signalling through quorum sensing. We conclude that there is a lack of evidence to support a specific signalling mechanism and a critical signal concentration of these behaviours in *S. cerevisiae* and thus these features require further investigation.

Oral abstracts

SESSION: YEAST

[O09] IDENTIFICATION AND SUCCESSION OF SPOILAGE YEASTS IN THE DANISH FETA-TYPE CHEESE

Athina Geronikou[1], Nadja Larsen[1], Søren Kristian Lillevang[2], Lene Jespersen[1]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] Arla Innovation Centre, Aarhus, Denmark

The Danish feta-type cheese is a soft, white-brined cheese produced from bovine milk and ripened in brine solution. Yeasts are major spoilage microorganisms in feta-type cheeses causing blemishes and off-flavors in the final products and, thereby, quality loss and shelf-life reduction. The aim of the present study was to perform taxonomic characterization of contaminating yeasts in the Danish feta-type cheese and investigate succession of yeast species during the shelf-life of the cheese product. In total, 798 yeast isolates were purified from the white-brined cheese incubated at 5°C and 10°C for 52 weeks. Viable counts of yeasts during incubation were generally increased from 3 to 7 log CFU/g and overall, these high levels remained till the 52nd week. Isolated yeasts were characterized for their macro- and micro-morphology, classified genotypically using (GTG)5-PCR fingerprinting, and identified by sequencing of the D1/D2 region of the 26S rRNA gene. The dominating yeasts species belonged to the genera *Candida* and *Debaryomyces*, primarily *C. zeylanoides* and *D. hansenii* (53% and 24% of all the isolates, respectively). The less frequently isolated yeasts were identified as *Kazachstania bulderi*, *Kluyveromyces lactis*, *Pichia* spp., *Rhodotorula mucilaginosa, Toluros-pora delbrueckii,* and *Wickerhamonyces anomalus*. Samples incubated at 10°C were characterized by higher diversity of yeasts (4 - 9 species) and surprisingly slightly lower viable counts compared to 5°C (2 – 5 species). The study emphasizes that taxonomic heterogeneity, rather than contamination levels, is an important factor influencing the product quality at storage. The knowledge on taxonomy and occurrence of spoilage yeasts in the Danish feta-type cheese will allow the dairies to do a knowledge-based control of contaminating yeasts and ensure extended shelf-life of the dairy products.

SESSION: WHOLE GENOME SEQUENCING

[O10] METAGENOMICS FOR FOOD AUTHENTICITY, DISEASE, HEALTH AND SURVEILLANCE

Frank Aarestrup[1]

[1]Technical University of Denmark, Denmark

Next-generation sequencing (NGS) has drastically changed our abilities to determine the complete DNA-content in any given sample or bacterial isolate. NGS is today or soon expected to be routinely used for human precision medicine, identification and tracking of pathogenic bacteria and basic understanding of metabolic processes in any organism.

However, when applied on mixed samples, so-called metagenomics, the use of NGS also has major potential as well as additional challenges. This involves challenges both in relation to sensitivity and specificity of identification of organisms and genes, as well as computational challenges in mapping DNA-sequences to large databases, as well as the basic handling and biological understanding of the extreme amount of data.

I will present example on how the GenEpi group at DTU is trying to utilize NGS and metagenomics for different purposes, ranging from food authenticity to global surveillance. This will also include discussion on the potential benefits, as well as the challenges.

SESSION: WHOLE GENOME SEQUENCING

[O11] *IN SILICO* PREDICTION OF APPLICATION-RELEVANT PHENOTYPES BASED ON WHOLE-GENOME SEQUENCES

Kristian Jensen[1]

[1] Computational Systems Biology, Discovery, R&D, Chr. Hansen A/S, Denmark

Through continuous advances in sequencing technologies, microbial whole-genome sequences of increasing quality can be obtained at decreasing cost. While the availability of whole-genome reference sequences for many microbial organisms has greatly benefited research and contributed to the understanding of microbial physiology, the technology now allows us to routinely sequence every individual strain. This increased level of detail in genetic data opens up new avenues for scientific research and development, including the discovery of the genetic basis for strain differences as well as quick prediction of relevant phenotypes based on genomic content.

Here we present examples of how we leverage the value of thousands of whole-genome sequences from the Chr. Hansen Culture Collection to guide the development of new products with improved application characteristics. By correlating the genome sequences of hundreds of Lactococcus lactis strains with measurements of milk acidification rates, we developed a machine learning model that can accurately predict the milk acidification rate based on the presence of specific protein domains in a strain. Similarly, correlating the presence of orthologous gene groups in strains of Streptococcus thermophilus with measurements of end point viscosity in milk fermentations allowed us to predict each strain's texturing ability for yogurt applications. These two proofs of concept demonstrate the theoretical feasibility of designing cheese and yogurt cultures in silico, with desired acidification and texturing characteristics. Additionally, we have analyzed over a thousand Bacillus genomes to identify strains with inhibitory bioactivity against plant pathogens. In vitro screening results indicated that strains selected based on comparative genomics analyses and in silico predictions of secondary metabolite clusters show significant inhibitory activity.

SESSION: WHOLE GENOME SEQUENCING

[O12] DECODING THE EFSA REQUIREMENTS FOR MICROBIAL SAFETY AND THE TECHNICAL CHALLENGES

Adalberto Costessi[1], Derek Butler[1], Radhika Bongoni[1], Danny Duijsings[1], Tom Van Den Bogert[1]

[1] Baseclear Bv, Leiden, Netherlands

Microorganisms have a long history of use in the food and feed industry across the world. Bacteria, yeasts and filamentous fungi are often used as food and feed additives or as production organisms for biomolecules like vitamins and enzymes. The European Union and other countries require a safety assessment and formal authorisation procedure before these products can be placed on the market.

In the last twenty years, advances in DNA sequencing technologies have impacted and accelerated developments in the food and feed industry. Importantly, genome sequencing technologies combined with advanced bioinformatic tools play also an increasingly important role in the safety assessment. Notably, the further development of genomic technologies is also enabling the governing bodies to crystallize their requirements.

We will discuss current requirements for EFSA safety assessment and technical approaches. Technical as well as regulatory challenges will be presented: examples include the availability of good databases for genome analysis, and the challenges of less standard microorganisms with increasing interest like algae and bacteriophages.

Oral abstracts

SESSION: WHOLE GENOME SEQUENCING

[O13] *PHAEOBACTER INHIBENS*, A PROBIOTIC TO CONTROL BACTERIAL INFECTIONS IN AQUACULTURE

Eva Sonnenschein[1], **Mathieu Castex**[2], **Lone Gram**[1]

[1] *Technical University of Denmark, Denmark*
[2] *Lallemand., France*

Fish farming is an essential food production to supply protein for the growing world population. However, in intense fish rearing systems, pathogenic bacteria spread rapidly and one particularly sensitive stage is the larval development. To avoid antibiotics to control infections and subsequent spread of antibiotic resistance, proliferation of bacterial pathogens can be suppressed by addition of probiotic bacteria. The marine alphaproteobacterium *Phaeobacter inhibens* has demonstrated potential as aquaculture probiotic and this study provides an overview on its genetics and physiology and the current status on safety of *P. inhibens* for future application in aquaculture industries. *P. inhibens* belongs to the widespread and environmentally important *Roseobacter* group and strains have been isolated globally from coastal waters and marine aquaculture facilities. *P. inhibens* inhibits or even kills many fish pathogenic bacteria such as *Vibrio*. It maintains this activity also in the presence of aquaculture-relevant feed organisms and fish larvae and causes the reduction of *Vibrio* by typically 2 to 4 log. In nature, *Roseobacter*–group strains are adapted to interactions with higher organisms and *P. inhibens* has no or a stimulating effect on higher organisms such as aquaculture live feed or fish larvae. Maintaining a balanced microbiome in all trophic levels is key to the sensitive, high intensity fish rearing systems and addition of *P. inhibens* caused only minor changes to the microbiome of live feed. The probiotic activity of *P. inhibens* is primarily due to the production of the antibacterial compound tropodithietic acid (TDA) and resistance to TDA could not be induced in pathogenic vibrios. The genome of *P. inhibens* encodes secretion proteins and proteases and the species is capable of quorum sensing, biofilm formation, and motility. Based on the great potential of *P. inhibens* to effectively combat fish pathogens without causing resistance development, its stable performance in live feed and fish larvae challenge trials and no adverse effect to the host, its minor effect on the host-associated microbiome and its natural occurrence, the probiotic *P. inhibens* could present an environmentally sustainable and economical solution to counteract the economic loss caused by bacterial pathogens in aquaculture systems.

SESSION: METABOLIC MODEL

[O14] MODELING APPROACHES FOR UNDERSTANDING FOOD FERMENTATIONS

Bas Teusink[1], **Douwe Molenaar**[1], **Bob Planqué**[1], **Herwig Bachmann**[1], **Frank Bruggeman**[1]

[1] *Systems Biology Lab, The Netherlands*

Microorganisms have to cope with different environments, either predictable ones caused by the organism's own metabolism -such as diauxie- or imposed transitions in nutrient availability or stresses. We study the regulation of metabolism from such an adaptive perspective, at different time scales. At short time scale, signaling and metabolic regulation steers the dynamics of metabolism towards desired flux states. At longer time scales gene expression regulation steers protein levels towards adapted states. We have developed theory that defines such protein expression states based on optimal allocation of limited cellular resources for maximal specific growth rate. One conclusion is that the degrees of freedom in the optimal flux state is bounded by the number of growth-limiting constraints on the proteome. I will illustrate the use of these insights through comparisons between experimental data and comprehensive models of metabolism and protein expression, in *Saccharomyces cerevisiae* and *Lactococcus lactis*.

SESSION: METABOLIC MODEL

[O15] REVISITING A CLASSIC BIOLOGICAL PHENOMENON - THE MICROBIAL LAG PHASE

Lieselotte Vermeersch[1,2], Gemma Perez-Samper[1,2,3], Bram Cerulus[1,2], Abbas Jariani[1,2], Brigida Gallone[1,2,3], Andrea Del Cortone[1,2], Supinya Piampongsant[1,2,3], Karin Voordeckers[1,2,3], Jan Steensels[1,2,3], Kevin J. Verstrepen[1,2,3]

[1]VIB – KU Leuven Center for Microbiology, Belgium
[2]CMPG Laboratory of Genetics and Genomics, KU Leuven, Belgium
[3]Leuven Institute for Beer Research (LIBR), Belgium

When faced with environmental changes, microbial cells enter a lag phase during which the cell cycle is arrested to allow cells to adapt to the new situation. The discovery of this lag phase started the field of gene regulation, and ultimately led to the unraveling of underlying mechanisms (and a Nobel Prize). In addition to being an interesting biological phenomenon, the lag phase also influences the efficiency of industrial fermentations, with longer lags leading to sluggish or stuck fermentations. Surprisingly, however, the factors that determine the exact duration and dynamics of the lag phase remain largely elusive.

Naively, one would expect that cells adapt as quickly as possible, so they can resume growth and compete with other organisms. However, our results show that the lag phase can last from several hours up to several days. Moreover, some cells within the same population take much longer than others, despite being genetically identical. In addition, we found that the duration of the lag phase is also influenced by the past, with recent exposure to a given environment leading to a quicker adaptation when that environment returns. Genome-wide screens suggest that the length of the lag phase, the heterogeneity in lag times of individual cells, and the history-dependent behavior are not determined by the time it takes to induce a few specific genes related to uptake and metabolism of a new carbon source. Instead, a major shift in general metabolism, and in particular a switch between fermentation and respiration, is the major bottleneck that determines lag duration. Interestingly, there is also a genetic component to lag duration, as some yeast strains show longer average lag times compared to others. QTL analysis suggests that an as yet uncharacterized gene is at the heart of these strain-specific differences, and that manipulating this gene might increase the industrial performance.

Together, our results show that the lag phase is a complex phenomenon that is influenced by both genetic and epigenetic mechanisms that together coordinate transcriptional and metabolic reprogramming. Interestingly, the duration of the lag phase differs greatly between genetically indentical cells as well as between genetically distinct populations, suggesting that the duration of the lag phase may involve a bet-hedging startegy shaped by evolutionary forces.

SESSION: METABOLIC MODEL

[O16] UNDERSTANDING THE INFLUENCE OF FERMENTATION NUTRIENTS AND (SHORT) PEPTIDES IS CRITICAL FOR DEVELOPPING EFFECTIVE FOOD CULTURES AND PROBIOTICS INDUSTRIAL MANUFACTURING

Alain M. Sourabié[1]

[1] Procelys BY Lesaffre, Maisons-Alfort, France

The industrial fermentation of food cultures and probiotic is usually performed in semi-defined or complex media which composition is of paramount importance to reach high levels of active biomass. From a manufacturer perspective, key targets when producing cultures and probiotics include growing them to high cell concentration while minimizing cells viability lost during stabilization. Several ingredients are available for industrial manufacturing, particularly yeast extracts and more recently yeast peptones (YE, YP) providing a wide variety of nutrients such as nitrogen components, vitamins and trace elements. Peptides uptake and utilization have been widely reported to be critical for the nutrition of industrially relevant micro-organisms.

In this study, we specifically aimed at exploring the nitrogen-based content and evaluating YE and YP nutritional and regulatory role during the fermentation process of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. A comprehensive analytical pipeline that combines a mass-spectrometry based peptidomic approach for mapping YE and YP peptides as well as a bottom-up proteomics and RNAseq to evaluate strains metabolic and physiological needs was therefore set-up. About 4,600 different oligopeptides ranging from 6 to more than 30 amino acids in length were subsequently identified and clustered to decrypt the rules of peptide utilization during fermentation. The physicochemical characteristics of consumed peptides perfectly matched the known affinities of the oligopeptide transport system of *S. thermophilus* for instance. Moreover, we were able to demonstrate that net charge is the major factor for oligopeptide transport in *S. thermophilus*. These peptides utilization dynamics was in addition shown to reflect the activity and the specificity of the oligopeptide transporter *Ami*.

Besides, experiments to evaluate growth rate, acidification kinetics and biomass enhancement were performed on *L. acidophilus* an *S. thermophilus* in bioreactors yielding substantial results on all parameters analyzed. The result of viable cells count and vitality measurement carried out using both flow cytometry and classical plating strongly indicated the benefits of combining both YE and YP to produce these strains.

Finally, the strategy used and the methodologies developed enable to better understand the adequacy between YE and YP composition and their beneficial impact on cells growth, fitness and metabolic activity.

SESSION: METABOLIC MODEL

[O17] CONTRAINT-BASED METABOLIC MODELING IN MICROBIAL FOOD BIOTECHNOLOGY: *STREPTOCCUS THERMOPHILUS* AS A CASE STUDY

Martin Holm Rau[1]

[1] Chr. Hansen, Denmark

The application of a constraint-based modeling (CBM) frameworks to genome-scale metabolic models (GEM) enables both the qualitative and quantitative analyses of the entire metabolism of an organism. Within the area microbial food biotechnology, CBM can benefit both the industrial production of microbial food cultures and their application in food processing. For example, CBM can be employed for providing mechanistic understanding of microbial physiology, guiding rational strain improvement and design of food cultures as well as enabling knowledge-driven bioprocess optimization for industrial-scale production of these cultures.

Streptococcus thermophilus is a key organism within the dairy industry as a starter culture for yoghurt and cheese production. Here, we applied CBM for obtaining novel insights into the key physiological characteristics of this organism. A refined genome-scale metabolic model that accurately could represent the metabolism of an industrial *S. thermophilus* strain was initially constructed. A CBM framework was applied to achieve a mechanistic understanding of secretion product profile variation by revealing an intricate relationship between redox balance and secretion levels of the key metabolites lactate, formate, acetoin and acetaldehyde.

The findings obtained here through constraint-based modeling hold the potential to guide rational strain improvement towards a metabolite secretion profile tailored to specific food applications.

PLENARY SESSION: FUTURE TRENDS AND REGULATORY AFFAIRS: CHALLENGES AND OPPORTUNITIES

[O18] GETTING BACK TO THE ROOTS OF FERMENTATION: PRESERVING FOOD AND EXTENDING SHELF LIFE

Francois Bourdichon[1]

[1] Università Cattolica del Sacro Cuore, France

Today's food industry is looking for means of producing safe food products with adequate shelf life, thus reducing food waste and meeting the consumer demands for reduced use of preservatives. As a human food processing technique, fermentation is the oldest food preservation technique going back to Neolithic times.

Fermentation is an ancient technology to serve food, preserve food and increase its health benefits.

While the traditional use of cultures in fermented foods refers to their positive action on product properties (texture, aroma, digestibility, nutritional values, aspect ...), the use of food cultures can inhibit the spoilage microflora and improve food safety. Fermentation and the resulting protection can be ascribed to several biological mechanisms including the ability of food cultures to:

- Control acidity of the food material as a natural consequence of fermentation
- Produce metabolites, enzymes and various naturally occurring compounds or degradation products from the food product such as peptides derived from proteolysis of food proteins
- Compete with other microorganisms, e.g. through ecological competition for limited nutrients, oxygen, space in the food product or head space in the food container, interaction with quorum sensing, destruction of harmful biofilms.

Bioprotection therefore refers to enhanced food safety and extended shelf life of foods by indigenous and/or intentionally added microflora, with their microbiological competition and production of antimicrobial metabolites to help inhibit the growth of pathogens and spoilage microorganisms.

Bioprotection enhances the effectiveness of a food management system but is never an alternative to good cleaning practices, hygienic design of the production and cold chain conservation. It adds on an extra hurdle for perishable food products.

Food cultures used directly in food production are regarded as food ingredients in the majority of the World. As bioprotection is rediscovered for its role in food processing, regulatory aspects can become a hurdle for practical application in the food chain as bioprotective cultures can wrongly be considered as "new" food additives by regulatory agencies.

Oral abstracts

PLENARY SESSION: FUTURE TRENDS AND REGULATORY AFFAIRS: CHALLENGES AND OPPORTUNITIES

[O19] THE LEGAL STATUS OF MICROBIAL FOOD CULTURES IN THE EU: A PRACTICAL GUIDANCE FOR RESEARCH INSTITUTES & THE FOOD INDUSTRY

Bernd van der Meulen[1], Gilles Boin[2], Caroline Herody[3]

[1] *European Institute for Food Law*
[2] *Product Law Firm*
[3] *Chr. Hansen*

The use of microorganisms is one of the oldest food processing technologies resulting in the transformation and preservation of food. In the EU, microbial food cultures (MFC) preparations composed of species with a long history of safe consumption are considered as "food" legally permitted for use in human food without pre-market authorization.

MFC with a technological impact on food are called "starter cultures". They may be present as natural microflora in the food, or as a result of the intentional addition of the MFC in an industrial food fermentation process. MFC preparations have always been used as ingredients "considered as foods which may be used for a technological function" to replace the spontaneous flora by safe known microorganisms providing the desired effects (preservation, texture, color, taste).

MFC that are intentionally added to both fermented and non-fermented foods (incl. food supplements) for their beneficial effect on consumers' health are called "probiotics".

Consumption of foods processed with the addition of MFC is one of the key consumer trends globally, resulting in an increasing number of those foods, some consisting of new recipes, in the offering of both retailers & restaurants.

Even though the legal status of MFC in the EU, along with data required to substantiate their safety, has been addressed in several publications, debates are still ongoing on criteria to be met by those ingredients, especially when used in new recipes, to be considered as "food" vs. having to be classified as a "food additive" or as a "novel food".

In this context, a multidisciplinary group of professionals, bringing expertise in science, law and food processing has been developing, based on an in-depth analysis of the European legal acts governing the use and labelling of foods, a guidance to research institutes & the industry for determining the conditions under which a certain MFC/recipe combination should be considered as a "food".

The outcome of this work is a follow up of a first analysis performed ten years back and published in the European Food & Feed Law review (Herody et. al, 2010)

SESSION: ENZYMES FOR FOOD AND FEED APPLICATIONS

[O20] ENZYME APPLICATION IN FOOD

Jens Frisbæk Sørensen[1]

[1] *Iff, Denmark*

Enzyme application in food has a long history. Over the last several decades a wide range of enzymes have been applied in various food applications. The main objective for using enzymes has been to facilitate industrial manufacturing of food and to overcome some of the natural raw material variations which have always challenged this industry. However, the consumer acceptance of various food ingredients, the realization of how foods influence our health and the changed view on sustainability and how we manage our global resources has opened for a much more sophisticated application of enzymes in food. I will give an overview of the enzymes currently used in the food industry, their functionality and some of the novel applications.

SESSION: ENZYMES FOR FOOD AND FEED APPLICATIONS

[021] ENZYME APPLICATIONS IN FEED

Albert van Dijk[1]

[1]Schothorst Feed Research, Netherlands

Enzymes are widely used in the feed of livestock, mainly in poultry and pigs. Enzymes added to feed can be regarded as a supplementation to the endogenous enzymes produced by the animal itself. In most cases, the supplemented enzymes are not produced by the animal. These enzymes are produced biotechnologically by bacteria or yeasts, and the main ones are: phytase, cellulase, xylase, glucanase, amylase, protease, mannanase and galactosidase. The main reason for enzyme application in animal feed is improving the digestibility of the feed. This increases the nutritional value of the feed with regard to absorption of minerals, energy and/or amino acids. As a result, growth performance and feed efficiency will increase and although depending on the market prices feed costs can be reduced. Intestinal health may also improve and excretion of nitrogen and phosphorus in the environment can be reduced. Phytase is used on a large scale since the nineties and is the most successful commercial enzyme in practice. Phytase releases phosphorus, calcium and amino acids bound to phytate molecules in vegetable feedstuffs and hence increases the availability of these nutrients for the animal. In poultry, NSP-ases are generally used in diets with high amounts of cereals . These enzymes break down Non-Starch Polysaccharides (NSP's) hereby reducing the viscosity of the content of the gastro-intestinal tract, resulting in a better growth performance, improved feed efficiency, a better litter quality and less dirty eggs.

Some more recent applications are proteases that might improve protein digestibility and enzymes that break down mycotoxins. Current research programs aim at developing more effective and/or more stable prototypes of the above mentioned enzymes. Enzyme applications in rations for ruminants are investigated as well. Also, research programs are running with enzymatic pre-treatment of raw materials, in order to break down lignocellulosic cell wall structures to release nutrients and hence improve the feeding value of these feedstuffs. This is often done in combination with heat- and/or mechanical pre-treatment. In the future this pre-treatment with enzymes of by-products available might help to promote circular economy and increase the nutritional values of these feedstuffs without penalizing the performance of the animals and improving the animal health and behaviour. Finally this application might also help to reduce the environmental impact of animal production.

SESSION: ENZYMES FOR FOOD AND FEED APPLICATIONS

[022] SUBSTRATE SPECIFICITY OF EXTRACELLULAR SERINE PROTEINASES FROM LACTIC ACID BACTERIA

Egon Bech Hansen[1]

[1]National Food Institute, Technical University of Denmark, Lyngby, Denmark

Lactic acid bacteria (LAB) are typically auxotrophic for several amino acids and depend on external supply of peptides and amino acids. If the environment does not contain sufficient low molecular weight nitrogen to support growth, LAB need a proteolytic system to use available proteins as a source of nitrogen.

Extracellular cell wall anchored proteinases are common in LAB. These cell envelope proteinases (CEP) are serine proteases with an architecture consisting of a proteinases domain with similarity to the bacillus protease subtilisin. However, with a PA-domain inserted into the proteinase domain. The proteinase domain is followed by domains of unknown function and ending with an anchor domain recognized by a sortase, which links the CEP covalently to the peptidoglycan of the cell wall.

The proteinases of this common architecture differ in substrate specificity ranging from proteases with only one known substrate to proteases cleaving the substrates at multiple sites.

The structure of each domain of a *Lactococcus* CEP was modelled from the amino acid sequence and assembled to a predicted model for the entire CEP. The predicted structure suggests a mode of action for this class of enzymes where the specificity of the CEP enzymes might be determined by a fibronectin like domain interacting with the protease domains rather than complementarity at the active site.

This model let us to reinvestigate the specificity of the PrtP enzyme from dairy strains of *Lactococcus lactis.* Isogenic strains of *L. lactis* strain MG1363 carrying six different PrtP enzymes (NCDO712, Wg2, HP, SK11, MS22333, and MS22337) were constructed. Two versions were constructed for each enzyme; one version anchored to the cell wall (wt), and an unanchored truncated version. The peptides generated by fermentation in milk were analyzed by proteomic analysis by HPLC-MS. Two types of milk were used, cow milk and camel milk. Analysis of the length of peptides and the observed cleavage sites allow us to conclude that the actual cleavage site is rather unspecific, whereas there is a discrimination against cleavage near the ends of the substrates, and some selectivity among the protein substrates.

[O23] CHARACTERIZING PATATIN SPECIFIC PROTEASE ACTIVITY BY HIGH-THROUGHPUT HOMO-FRET ASSAY AND MASS SPECTROMETRY

Lise Friis Christensen[1], Simon Gregersen[2], Michael Toft Overgaard[2], Egon Bech Hansen[1]

[1] Technical University of Denmark, Denmark
[2] Aalborg University, Denmark

Food waste and industrial side streams with high protein contents are potential and widely unexplored sources for new food ingredients. In particular, peptides liberated by proteolysis may hold unidentified latent bioactivities. However, protease selection for this liberation process is extremely challenging, as liberation of target peptides will require specific proteolysis. Microbes are experts in spontaneous food fermentation why they may contain proteases with desired functionalities. Auxotrophic lactic acid bacteria (LAB) have adapted to diverse nutrient rich environments where they require peptide availability and uptake. Interestingly, extracellular proteases of some LABs generate sufficient peptide supply by hydrolyzing surrounding proteins, but they often display high substrate selectivity. For instance, extracellular proteases of *Lactococcus lactis* are highly selective towards certain caseins. Screening other microbes may reveal novel proteases with new desired specificities that may be substantiated further through bioengineering. Nevertheless, identification of proteolysis specific for certain proteins or peptide products will require efficient high-throughput screening assays to characterize new proteolytic activity.

Here, we present a fluorescence-based assay to measure proteolysis of the main potato protein, patatin, which was recently shown to embed emulsifying and antioxidant peptides. This patatin-specific assay detects protease activity with high sensitivity by measuring the increase in fluorescence emission. Emission increases due to the decreased homo-FRET (fluorescence resonance energy transfer), which results from proteolysis of extensively labelled patatin. The assay can easily be performed in a microplate format to achieve reproducible, high-throughput screening of proteolytic activity. We have validated the microplate format of the assay, which detects patatin-specific proteolytic activity efficiently by a variety of commercial proteases. We investigated the effect of protein labelling on proteolytic activity by comparing proteolysis of labelled and non-labelled patatin. We show that our approach combining high-throughput homo-FRET assays and mass spectrometry are useful to characterize specific activity and cleavage patterns of isolated proteases *in vitro*, which can easily be adapted for other proteases and substrates. Our vision is to adapt this assay for screening of cell cultures to facilitate characterization of e.g. membrane-bound extracellular protease activity.

SESSION: MICROBIALLY DERIVED BIOACTIVE COMPOUNDS

[O24] THE BIOTIC FAMILY DEFINITIONS AND FUTURE RESEARCH PERSPECTIVES

Seppo Salminen[1]

[1]FFF, Faculty of Medicine, University of Turku, Finland

Definitions are important for science, regulations and the whole society and therefore the International Scientific Society of Probiotics and Prebiotics (ISAPP) has had a long-standing mission of producing science-based open access consensus definitions for microbiota modulating agents such as probiotics and recently also fermented foods.

Gut microbiota influences health and impacts our metabolism in part by interacting with our dietary components and converting them into metabolic products. Microbiota-targeted dietary components include the `biotic` family, probiotics, prebiotics, synbiotics and postbiotics as well as fermented foods. The first gut microbiota modulating substances may have been fermented foods. Fermented foods and beverages have a long history of use, accompanying the transition from hunter-gatherer communities to agricultural communities in the Neolithic revolution about 14,000 years ago. Fermented foods may theoretically encompass all these substances, as they supply us with microbes at various stages along the live-dead continuum, predigested nutrients, and bacterial metabolites, all of which may affect human gut microbiota.

Understanding fermentation processes has changed our perceptions of the components in and the nutritional value of fermented foods, leading to the idea of 'biotic' components in our foods with potential to modulate microbiota. Facilitating such processes may lead to the formulation of effective foods with 'biotic' components able to positively impact gut microbiota development and health from infants to the elderly. Biotic components are defined by the Nature journals: Biotic components are the living organisms present in an ecosystem, such as bacteria, fungi, plants and animals, and elements produced by them (https://www.nature.com/subjects/biotic). These offer novel possibilities for future products.

Fermented Foods	Foods made through desired microbial growth and enzymatic conversions of food components	Hill C et al Nat. Rev. Gastroenterol. Hepatol. 11, 506–514 (2014)
Probiotic	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host	Gibson G et al Nat. Rev. Gastroenterol. Hepatol. 14, 491–502 (2017)
Prebiotic	A substrate that is selectively utilized by host microorganisms conferring a health benefit	Swanson K et al Nat. Rev. Gastroenterol. Hepatol 17, 687–701 (2020)
Synbiotic	A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host	Marco M et al Nat. Nat. Rev. Gastroenterol. Hepatol 18, 196-208 (2021)
Postbiotic	Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host	Salminen, S. et al. Nat Rev Gastroenterol Hepatol, 18, 649-667 (2021)

Table 1. ISAPP consensus definitions for fermented foods, probiotics, prebiotics, synbiotics and postbiotics.

Oral abstracts

SESSION: MICROBIALLY DERIVED BIOACTIVE COMPOUNDS

[O25] ENGINEERING YEAST FOR FEED AND FOOD INGREDIENTS

Irina Borodina[1], Steven van der Hoek[1], Behrooz Darbani[1], Karolina Zugaj[1], Bala Krishna Prabhala[1], Mathias Bernfried Biron[1], Milica Randelovic[1], Jacqueline Medina[1], Douglas Kell[1]

[1] The Novo Nordisk Foundation Center for Biosustainability, Denmark

Various food, feed, agriculture, and pharma ingredients can be produced via yeast fermentation, at a low cost and with a better sustainability profile than via extraction from natural sources. I will share our work on engineering baker's yeast *Saccharomyces cerevisiae* and oleaginous yeast *Yarrowia lipolytica* to produce L-(+)-ergothioneine, resveratrol, natural colors, and psilocybin. Ergothioneine is an antioxidant present in many edible mushrooms. In animal models, it showed promise against neurological diseases, pre-eclampsia, and some cardiometabolic disorders. Resveratrol is an antioxidant from grapes. Psilocybin is a psychoactive alkaloid present in so-called "magic mushrooms," currently investigated to treat depression, anxiety, and clustered headache.

References:

Milne et al (2020) "Metabolic engineering of *Saccharomyces cerevisiae* for the *de novo* production of psilocybin and related tryptamine derivatives". *Metab Eng*, 60:25-36.

van der Hoek et al (2019) "Engineering the yeast *Saccharomyces cerevisiae* for the production of L-(+)-ergothioneine". *Front Bioeng Biotechnol*, 7:262.

Sáez-Sáez et al (2020) "Engineering the oleaginous yeast *Yarrowia lipolytica* for high-level resveratrol production". *Metab Eng*, 62:51-61.

Tramontin et al (2019) "Enhancement of astaxanthin biosynthesis in oleaginous yeast *Yarrowia lipolytica* via microalgal pathway". *Microorganisms,* 7(10):472.

SESSION: MICROBIALLY DERIVED BIOACTIVE COMPOUNDS

[O26] MICROBIAL PROTEINS: MOVING FROM FEED TO FOOD APPLICATIONS AIDED BY PROTEOMICS AND BIOINFORMATICS

Simon Gregersen[1], Betül Yesiltas[2], Pedro Jesus Garcia Moreno[3], Tobias Hegelund Olsen[4], Paolo Marcatili[2], Charlotte Jacobsen[2], Egon Bech Hansen[2], Eleni Ntokou[5], Ib Christensen[5], Michael Toft Overgaard[1]

[1] Aalborg University, Aalborg, Denmark
[2] Technical University of Denmark, Denmark
[3] University of Granada, Spain
[4] Oxford University, United Kingdom
[5] Unibio, Odense, Denmark

Fermentation of bacteria has within the last decade received an increasing amount of attention as a sustainable production method for feed protein. In particular, the use of methanotrophic bacteria such as *Methylcoccus capsulatus* has been shown to be an excellent example of how microbial fermentation can convert greenhouse gasses such as methane into a highly valuable resource. The resulting biomass has a very high protein content as well as an excellent nutritional profile and, importantly, can be produced in a scalable and financially profitable manner. The application of this protein-rich biomass in foods or food ingredients remain largely unexplored, but could be a potential part of the solution for higher global demand of sustainable protein. A critical aspect in the applicability is the modest characterization of the protein-level functionality and how processing affect the proteins and their properties on both the bulk and the molecular level.

In this work, we show how applying a combination of proteomics and bioinformatic analysis is an extremely powerful tool for addressing these questions. Performing deep proteome characterization using LC-MS/MS-based proteomics, we have been able to quantitatively identify the most abundant proteins in the crude fermented biomass. By applying novel bioinformatic tools, we have subsequently been able to identify embedded peptides with desirable functional properties. Using *in vitro* assays, we were indeed able to verify that the biomass is a source of proteins with embedded peptides displaying emulsifying and antioxidant properties. Through sequence analysis, we show how the peptides may be obtainable using targeted, enzymatic hydrolysis. Lastly, we demonstrate how combining existing protein annotation and bioinformatic prediction of subcellular localization can be applied for evaluating lab- and pilot scale cell lysis and protein extraction efficiency as well as the protein level effects of downstream processing.

SESSION: MICROBIALLY DERIVED BIOACTIVE COMPOUNDS

[027] POSTBIOTICS IMPACT MICROBIOTA, HOST BEHAVIOUR AND COLITIS - AN EXAMPLE USING HEAT-TREATED LACTOBACILLI

Alicja Warda[1], Colin Hill[2]

[1] Apc Microbiome Ireland, Cork, Ireland
[2] Department of Microbiology, University College Cork, Cork, Ireland

The potential impact of heat-treated bacteria, their metabolites and cell fragments on host health and microbiome composition is gaining interest in the scientific community. Heat-killed microbes have potential advantages in terms of use and safety over living organisms, including extended shelf life, no/low risk of infection in vulnerable populations, and less risk of translocation of genetic material, for sufficiently heated preparations. A number of initiatives have attempted to define and regulate these non-replicating preparations, in the same way that definitions and regulations have been established for probiotics and prebiotics. Hill et al (2010) have proposed a provisional definition of pharmabiotics as *'cells of human origin, or their products, with a proven pharmacological role in health or disease*.' While Collado et al (2019) have proposed a provisional definition of postbiotics as '*compounds produced by microorganisms, released from food components or microbial constituents, including non-viable cells that, when administered in adequate amounts, promote health and well-being*'. Such preparations have also been referred to as ghost-probiotics and paraprobiotics. In the current study, we aimed at evaluating the effect of a heat-treated fermentate containing two *Lactobacillus* strains and the bacterial metabolites produced during fermentation (here termed ADR-159), on microbiota composition, host health and behaviour in mice.

We used murine models to assess the effect of ADR-159 on behaviour, infection, development of colitis, inflammation and behaviour. Simultaneously ADR-159 supplementation was applied to batch fermentation models inoculated with human faeces. Collected samples were analysed for microbiota composition, virome composition and short chain fatty acids.

ADR-159 elicited subtle yet significant microbiota modifications in both models, with no detrimental health effects in mice. The ADR-159 diet in animals led to increased sociability and lower corticosterone baseline levels. ADR-159 also prevented the damaging effects of the colitis, which results from *Citrobacter*-induced inflammation in animals.

This study demonstrates the impact of heat-treated bacteria and their metabolites on microbiota composition, on infection-induced inflammation and on the behaviour of healthy mice.

SESSION: MICROBIOME/ANIMAL & HUMAN HEALTH

[028] STEERING OF THE INTESTINAL MICROBIOTA COMPOSITION IN PRODUCTION ANIMALS, TO REDUCE *SALMONELLA* IN HUMANS

Filip Van Immerseel[1]

[1]Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases, Belgium

Salmonella is one the most important foodborne pathogens worldwide, and major sources are consumption of meat products and eggs. Vaccination has been successful in controlling egg contamination by *Salmonella* in laying hens, and as a result caused a serious reduction in human salmonellosis cases. Biosecurity measures and feed and drinking water additives have been the main tools to limit infection and colonization in broilers and pigs. In chickens, *Salmonella* is residing in the caeca, that are the fermentation chambers in the distal intestinal tract. It has been shown that butyrate and propionate reduce colonization of *Salmonella* because these short-chain fatty acids affect virulence gene expression and invasion of *Salmonella* in epithelial cells, what is essential for colonization. This opens perspectives for nutritional steering towards butyrate and propionate production in the caeca. The caeca contain a highly diverse microbiota composition, and numerous species of the highly abundant families *Lachnospiraceae* and *Ruminococcaceae* are anaerobic butyrate producers. The use of selected strains of these families as in-feed additives has been shown to affect *Salmonella* colonization, but these are anaerobic strains and thus difficult to develop as probiotics. Most commonly used probiotics do not affect *Salmonella* colonization although there can be exceptions. Prebiotic approaches that affect *Salmonella* colonization are described, and many are promoting butyrate formation in the intestinal tract (eg XOS, short-particle size wheat bran), while others might act through immunological mechanisms (glucans) or block adhesion to epithelial cells (MOS). All of these products will be able to reduce *Salmonella* colonization levels, but will not eliminate the bacterium from the gut. Instead of pre- and probiotic steering methods, also administration of butyrate formulations in feed has been shown to be effective. In the case of meat-producing chickens, the short life span, the high sensitivity post-hatch because of a lack of a microbiota, and the dense housing in floor pens, makes *Salmonella* protection through dietary interventions challenging.

SESSION: MICROBIOME/ANIMAL & HUMAN HEALTH

[029] INVESTIGATION AND MANIPULATION OF GUT MICROBIAL FUNCTIONS – THE EXAMPLE OF GLYCEROL/DIOL DEHYDRATASE ACTIVITY

Clarissa Schwab[1]

[1]Department of Microbiology, Nutrition and Dietics, Czech University of Life Sciences, Prague, Czech Republic

The human colon harbours an estimated number of 1011 bacteria/mL content. Fecal microbial gene catalogues predict the presence of about 800 000 genes/fecal sample, many of those might encode enzymes with unknown function. One family of enzymes expressed by gut microbes are cobalamin dependent glycerol/diol dehydrates, which metabolize the substrates glycerol and the fermentation intermediate 1,2-propanediol to 3-hydroxypropionaldehyde (3-HPA) and propanal, respectively. 3-HPA is a component of the reuterin system, which has antimicrobial activity and has been linked to the transformation of dietary carcinogenic heterocyclic amines. Acrolein, which forms spontaneously from 3-HPA, is the likely component of the reuterin system that is responsible for both bioactivities. Propanal can be further metabolized to the short chain fatty acid propionate, thus both metabolic activities of glycerol/diol dehydratases can be linked linked to intestinal health.

In recent studies, we investigated the potential of gut microbes selected based on genomic prediction to confer glycerol/diol dehydratase activity, and studied the potential and impact of microbial and nutritional manipulation on glycerol/diol dehydratase activity of complex intestinal microbial communities. Our results indicated that intestinal glycerol/diol dehydratase activity can be successfully manipulated, but that secondary – non beneficial – effects might occur

SESSION: MICROBIOME/ANIMAL & HUMAN HEALTH

[030] EVALUATION OF HUMIC ACIDS AS FUNCTIONAL FEED ADDITIVE ON PERFORMANCE, METABOLIC PARAMETERS AND GUT MICROBIOTA OF WEANED PIGLETS.

Matteo Dell'Anno[1], Stefania Sotira[2], Valentina Caprarulo[2], Monika Hejna[2], Serena Reggi[2], Francesco Miragoli[3], Maria Luisa Callegari[3], Aldo Prandini[4], Luciana Rossi[2]

[1] Università Degli Studi DI Milano, Milan, Italy
[2] Department of Health, Animal Science and Food Safety, Università Degli Studi DI Milano, Italy
[3] Università Cattolica del Sacro Cuore, Cremona, Italy
[4] Department of Animal Science, Food and Nutrition (Diana), Facoltà DI Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Italy

Humic acids (HA), originated from the decomposition of organic matter in the soil by microorganisms, have been recently added in the European catalogue of feed materials considering for their therapeutic effects on gastrointestinal diseases. In swine livestock, there is an urgent need of alternatives to antibiotics. Since the data present in literature are scarce and HA nutritional value is not well-defined, the aim of this study was to evaluate the *in vivo* effect of HA as functional dietary feed supplement in weaned piglets. One-hundred twenty weaned piglets (28±2 days) were randomly divided into two groups fed control diet (CON) and HA enriched diet (0.25%; HAG). Zootechnical parameters were measured during the entire experimental period (40 days). On individual faecal samples, gut microbiota composition was obtained by sequencing the V3-V4 regions of the 16S rRNA gene. Principal blood parameters were analysed by auto-analyser on individually collected serum (total protein content, albumin, globulin, albumin/globulin, urea, alanine aminotransferase, aspartate aminotransferase, phosphatase alkaline, total bilirubin, glucose, total cholesterol, calcium, phosphorus, magnesium, total triglycerides, high-density lipoproteins, low-density lipoproteins and creatinine).

Obtained results showed an increase of zootechnical performances (body weight, average daily gain, average daily feed intake) ($p < 0.001$) of the HAG compared to CON. The serum metabolic profile highlighted a decrease of total triglycerides ($p < 0.05$) with a higher content of cholesterol ($p < 0.05$) but only high-density lipoprotein revealed a rise ($p < 0.001$). An increase of observed species ($p = 0.01$) and Shannon's index ($p = 0.03$) was observed in HAG. Concerning β diversity, principal coordinate analysis (PCoA) plots revealed no distinct separation of samples. However, only a significant increase of *Prevotella* ($p = 0.035$) was detected indicating a very limited impact of this supplementation on intestinal microbiota.

In conclusion, HA included at 0.25% in the diet demonstrated a positive effect on growth rate and a better lipid profile suggesting that HA can be considered as innovative feed additive for weaned piglets.

Acknowledgements: The research study was done in the frame of FOODTECH PROJECT (ID 203370, this project is co-funded by European Regional Development Fund, ERDF).

Oral abstracts

SESSION: MICROBIOME/ANIMAL & HUMAN HEALTH

[O31] HIGH THROUGHPUT IN VITRO CHARACTERIZATION OF PECTINS FOR PIG(LET) NUTRITION AND HEALTH

Maria Wiese[1], Yan Hui[2], Jesper Holck[3], Jimmy J.P. Sejberg[4], Celia Daures[5], Evy Maas[6], Johanna Borne[4], Bekzod Khakimov[7], Thomas Thymann[8], Dennis Sandris Nielsen[2]

[1] Tno, Netherlands
[2] University of Copenhagen, Denmark
[3] Technical University of Denmark, Denmark
[4] Cp Kelco, Lille Skensved, Denmark
[5] Agrocampus, Department of Animal and Food Sciences, France
[6] Wageningen University, The Netherlands
[7] Department of Food Science, University of Copenhagen, Denmark
[8] Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Denmark

Pig production is facing challenges during post-weaning, such as high incidences of diarrhea accompanied by the current pressure from government authorities and consumers to replace antibiotics and zinc oxide. There is hence an elevated interest in the understanding of how fibre, such as pectin, can improve animal growth and health via direct or gut microbiota mediated effects. Pectin is a major building block of the plant cell wall and displays a plethora of molecular structures, it is a natural component of animal feed and the gastrointestinal tract and its microbiota co-evolved and adapted to its exposure. Animals vary in their carbohydrate degradation capacity depending on their age and the maturity of their gut microbiota. It is hence essential to unravel the mode of action of fibre and match it with specific animal target groups for an optimized effect on health and nutrition. In vitro colon models such as the CoMiniGut model operating at a low fermentation volume, requiring only small amounts of substrate are ideal for the investigation of the function of novel pectic substrates available in small quantities. In this study, we have investigated the effects of 20 substrates with defined molecular structure on freshly weaned piglet gut microbiota (GM) using the CoMiniGut. Various sugar beet derived pectin substrates such as high and low methylated pectin, rhamnogalacturonan-I (RG-I) and several oligosaccharides were investigated. For comparison, pectin structures from other plant sources such as pea and citrus pectin were included. We identified various structure-specific effects of the pectin substrates as well as differences in the effect of analog structures such as RG-I from different plant sources. Citrus RG-I promotes the growth of Lactobacillus within the piglet GM, whereby RG-I from sugar beet enhances growth of Bacteroides. Additionally, we investigated the effect of dosage and substrate mixtures on the GM in vitro and microbial community composition was furthermore correlated to short-chain fatty acid metabolites, known to play a role in health. In conclusion, our results show that pectin source and structure have a strong impact on GM composition in vitro, likely opening possibilities to direct the piglet GM in vivo.

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[O32] USE OF FUNGI TO CONVERT PLANT MATERIAL INTO HIGH QUALITY FOOD

Han Wösten[1]

[1]Microbiology, Department of Biology, Utrecht University, The Netherlands

Fungi colonize plant waste materials in nature. To this end, they secrete enzymes that degrade the substrate into low molecular weight molecules that can be taken up to serve as nutrients. As a result, polysaccharides in the waste material are being converted into fungal biomass that is rich in protein, fiber, vitamins, bioactive compounds and minerals. In addition, the structure of fungal biomass is similar to that of meat, making it an attractive meat replacement in our diet.

Mushroom production is based on conversion of low quality plant waste streams. Sales of mushrooms is increasing globally. Surprisingly, little is known about the processes that result in mushroom production. First, the vegetative mycelium has to colonize the substrate. Variables are the rate of colonization, density of colonization, and the enzymes that are secreted. This is followed by escape of hyphae into the air initiating the process of mushroom formation. We have studied transcription factors that are involved in this developmental process. By over-expression of the C2H2 regulator, formation of white button mushrooms accelerates. This shows that production of mushrooms can be improved in the future.

Our knowledge of colonization of substrates for mushroom production can also be used to create novel foods based on fungal colonization of high quality plant material such as oat and pea. Fungi are expected to improve digestability, protein composition, structure and flavor of such novel foods.

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[033] METABOLIC RESPONSE OF LACTIC ACID BACTERIA STRAINS DURING BREWERS' SPENT GRAIN FERMENTATION

Marta Acin Albiac[1], Rossana Coda[2], Carlo Giuseppe Rizzello[3], Pasquale Filannino[3], Marco Gobbetti[1], Raffaella di Cagno[1]

[1] Free University of Bozen-Bolzano, Italy
[2] University of Helsinki, Finland
[3] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Italy

The most abundant beer brewing by-product is brewers´ spent grain (BSG), which is the insoluble residue separated from the mash before fermentation. Its annual average global production is estimated to be ~39 million tons. BSG is mainly constituted by fibers (more than 50%, w/w) and protein (up to 30%, w/w). Currently, no recycling solution exists to exploit BSG by-products as food ingredient. We investigated the BSG niche-specific traits of selected lactic acid bacteria (LAB) strains through metabolomics, phenomics and differential gene expression. BSG from Finnish and Italian breweries were chosen as model systems, and MRS broth was used as a control. Growth kinetics, organic acids production, release of phenolic compounds and biomass dynamics were monitored at different time points. Switching of metabolism from MRS to BSG model media of selected LAB was investigated through Omnilog High Throughput Phenotype Microarray (Biolog). Phenotype data were analyzed using an in-house R pipeline and a Bayesian statistical approach was used to compute strain and media effect on the metabolism of different compounds. Highlighted putative metabolic pathways were studied through qPCR differential gene expression of LAB under both BSG and MRS model media. Findings showed a metabolism addressed towards the degradation of carbohydrate subunits naturally present in plant cell walls. Besides, β-galactosidase and phospho-β-glucosidases activities play an important role in the metabolism of sugar-modified phenols. Lactic acid fermentation may increase the bioavailability of health-promoting compounds as well modify BSG molecular structure, enhancing its nutritional and technological properties as a food ingredient. These *in vivo* phenomes investigation will enable a better establishment and understanding of tailored bioprocessing technologies for the exploitation of BSG as a food product through lactic acid fermentation.

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[034] BREWERS' SPENT GRAIN AS SUBSTRATE FOR SYNTHESIS OF DEXTRAN BY LACTIC ACID BACTERIA: REGULATION OF DEXTRANSUCRASES AND FERMENTATION PERFORMANCE

Prabin Koirala[1], Hanna Nihtilä[1], Ndegwa Henry Maina[1], Kati Katina[1], Rossana Coda[1]

[1] University of Helsinki, Finland

Dextran is a homopolysaccharide synthesized by lactic acid bacteria (LAB) by the action of extracellular dextransucrases in the presence of sucrose. Dextran offers texturizing, viscofying, and emulsifying functionality to food matrices, therefore, dextran synthesis *in situ* during LAB fermentation is a valuable means to enhance the properties and overall quality of many food products. Food materials or side streams with poor technological performance but with high nutritional properties, like legumes, bran, or brewers' spent grain (BSG), can be upgraded via dextran formation, becoming more suitable for food applications. Despite the growing interest in this technology, the regulatory mechanisms involved in dextran biosynthesis have not been extensively studied yet in industrially relevant conditions, such as during food transformation. Different environmental factors (e.g., substrate composition and cultivation conditions) can influence the microbial response and the biosynthesis of dextran, significantly affecting the yield and composition. In this study, the influence of BSG on the synthesis of dextran by *Weissella confusa* A16 and *Leuconostoc pseudomesenteroides* DSM 20193 was investigated. The performance of strains and the primary metabolites formed during fermentation were also analyzed.

Kinetics of bacterial growth, acidification, and viscosity change were followed during 24 h of BSG fermentation with and without 4% sucrose (w/w). A pH drop was observed after 10 h with significant changes at 16 and 24 h. Viscosity increased with the addition of sucrose, and particularly at 10, 16, and 24 h. Thus, these time points were selected for further analysis. Three different dextransucrase genes were identified in *L. pseudomesenteroides* DSM 20193 and one in *W. confusa A16.* Differential expression of dextransucrases was observed at the time points considered, and the presence of both dextran and isomaltooligosaccharides was confirmed.

The synthesis of dextran *in situ* in BSG using LAB fermentation is a promising technology that could contribute to reintroduce an underutilized food side stream into the food chain. Understanding how dextran is formed during fermentation could help to improve the fermentation process and its outcome and assist in the development of molecular approaches in food technology, enabling more efficient use of the resources.

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[035] LACTIC ACID BACTERIA FERMENTATION AS A TOOL TO IMPROVE THE ANTIOXIDANT PROPERTIES OF BREWERS' SPENT GRAIN: BIOPROCESS SET-UP, CHARACTERIZATION AND APPLICATION IN PASTA MAKING

Michela Verni[1], Rosa Schettino[1], Annika Krona[2], Rossana Coda[3], Carlo Giuseppe Rizzello[1]

[1] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari, Italy
[2] Rise, Research Institutes of Sweden
[3] University of Helsinki, Helsinki, Finland

Brewers' spent grain (BSG), the main by-product of the brewing industry, contains high concentration of fibers and polyphenolic compounds, which are mostly bounded to cell wall material. Despite its functional potential, BSG is used as low value feed or discarded as waste. Aiming at promoting its use in functional food industry, thirty-two LAB were screened for the ability of improving BSG antioxidant activity during fermentation. *Lactobacillus plantarum* PU1 and PRO17 and *Lactobacillus brevis* H46 caused the most relevant increases and were selected for further experiments. A biotechnological protocol for BSG treatment was set-up and optimized through the addition of a commercial xylanase. Bioprocessed BSG showed high inhibition of linoleic acid oxidation, comparable to that of BHT, and protective effect on human keratinocyte cell lines, comparable to that of α-tocopherol. The phenolic profile of raw and bioprocessed BSG was investigated through RP-HPLC-ESI-QTOF. Bound phenolic profile was characterized mostly by phenolic acids and their oligomers. Bound phenols in raw BSG resulted almost 50-fold higher than free phenols. The treatment with xylanase caused the release of 25% of the total bound phenols in free forms. An intense metabolic activity on phenolic acids was observed in fermented BSG leading to the increase of dihydrocaffeic and phloretic acids. Potentially bioactive peptides, showing the typical chemical features of antioxidant sequences, were purified and identified in BSG fermented with *Lb. plantarum* PU1. Peptides encrypted in native cereal proteins were released thanks to the proteolytic activity of the lactic acid bacteria used as starter for fermentation.

Bioprocessed BSG (bBSG), treated with xylanase and fermented with *Lb. plantarum* PU1, was used for pasta making, at 15% substitution level of semolina. bBSG pasta had high content of fibers (ca. 8%), with more soluble fibers compared to raw BSG pasta. An integrated approach including the determination of the main technological, nutritional, and sensory properties was used for the pasta samples characterization.

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[036] EVIDENCING FERMENTED RAPESEED MEAL USING LACTIC ACID BACTERIA IS AN ALTERNATIVE FOR DISCARDING HIGH DOSE OF ZINC OXIDE IN PIGLET PRODUCTION

Søren Kjærulff[2], Ninfa Rangel Pedersen[2], Gizaw D. Satessa[1], Paulina Tamez-Hidalgo[2], Yan Hui[3], Tomasz Cieplak[3], Lukasz Krych[3], Grete Brunsgaard[2], Dennis S. Nielsen[3], and Mette O. Nielsen[4]

[1]Department of Veterinary and Animal Sciences, University of Copenhagen, Denmark
[2]Fermentationexperts A/S, Copenhagen, Denmark
[3]Department of Food Science, University of Copenhagen, Denmark
[4]Department of Animal Sciences, Faculty of Science and Technology, Aarhus University, Denmark

Use of high doses of zinc oxide (ZnO, commonly termed medicinal zinc) in weaned piglet production will be phased out in Europe after 2022. There is currently no validated alternative to zinc for reducing diarrhea incidences and therefore, the use of prescription antibiotics. The present study investigated if lacto-fermented rapeseed meal (FRM), partly replacing soybean meal could promote similar performance in weaned piglets when medicinal zinc was omitted. Weaned piglets (n = 600) were tested from weaning and during the nursing period (28 – 85 days of age) with 3 feeding regimes, allocated in separate pens. A commercial basal diet with highly digestible soybean meal included at 8% (dry feed basis) was set as negative control (NC), same diet with additional 2500 ppm of ZnO was set as positive control (PC), basal diet with FRM included at 10% while soybean meal included at 4 % (dry feed basis) was the experimental diet. All diets were iso-caloric and iso-proteic. Piglets fed FRM had similar production performance compared to PC piglets. Jejunal villus development was stimulated over NC in PC and FRM. Furthermore, piglets from PC and FRM showed similar changes in composition and diversity of colon microbiota compared to NC piglets. In FRM piglets, the development of colon mucosal was stimulated greater than NC and PC with reduced signs of focal intestinal inflammation and health biomarkers in blood. Based on these results, we suggest FRM as a functional fermented protein meal with the ability of improving nutrition and health in weaned piglets without medicinal zinc.

Oral abstracts

SESSION: ENRICHMENT OF PLANT-BASED FOODS VIA FERMENTATION

[O36] LACTIC ACID BACTERIA FERMENTATION AS A TOOL TO IMPROVE THE ANTIOXIDANT PROPERTIES OF BREWERS' SPENT GRAIN: BIOPROCESS SET-UP, CHARACTERIZATION AND APPLICATION IN PASTA MAKING

Michela Verni[1], Rosa Schettino[1], Annika Krona[2], Rossana Coda[3], Carlo Giuseppe Rizzello[1]

[1] Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari, Italy
[2] Rise, Research Institutes of Sweden
[3] University of Helsinki, Helsinki, Finland

Brewers' spent grain (BSG), the main by-product of the brewing industry, contains high concentration of fibers and polyphenolic compounds, which are mostly bounded to cell wall material. Despite its functional potential, BSG is used as low value feed or discarded as waste. Aiming at promoting its use in functional food industry, thirty-two LAB were screened for the ability of improving BSG antioxidant activity during fermentation. *Lactobacillus plantarum* PU1 and PRO17 and *Lactobacillus brevis* H46 caused the most relevant increases and were selected for further experiments. A biotechnological protocol for BSG treatment was set-up and optimized through the addition of a commercial xylanase. Bioprocessed BSG showed high inhibition of linoleic acid oxidation, comparable to that of BHT, and protective effect on human keratinocyte cell lines, comparable to that of **α-tocop**herol. The phenolic profile of raw and bioprocessed BSG was investigated through RP-HPLC-ESI-QTOF. Bound phenolic profile was characterized mostly by phenolic acids and their oligomers. Bound phenols in raw BSG resulted almost 50-fold higher than free phenols. The treatment with xylanase caused the release of 25% of the total bound phenols in free forms. An intense metabolic activity on phenolic acids was observed in fermented BSG leading to the increase of dihydrocaffeic and phloretic acids. Potentially bioactive peptides, showing the typical chemical features of antioxidant sequences, were purified and identified in BSG fermented with *Lb. plantarum* PU1. Peptides encrypted in native cereal proteins were released thanks to the proteolytic activity of the lactic acid bacteria used as starter for fermentation.

Bioprocessed BSG (bBSG), treated with xylanase and fermented with *Lb. plantarum* PU1, was used for pasta making, at 15% substitution level of semolina. bBSG pasta had high content of fibers (ca. 8%), with more soluble fibers compared to raw BSG pasta. An integrated approach including the determination of the main technological, nutritional, and sensory properties was used for the pasta samples characterization.

2022 Belgrade

FEMS Conference on Microbiology

in association with **Serbian Society for Microbiology**

30 June - 2 July 2022 • Serbia

Sign up to our events newsletter and win a FEMS hoodie!

SCAN ME

Poster abstracts

SESSION: PRO-PREBIOTICS AND THE MICROBIOME

[P01] THE EFFECT OF DIRECT-FED MICROBIALS ON GRASS OR MAIZE SILAGE ON IN-INVITRO RUMEN FERMENTATION

Rajan Dhakal[1], Hanne Helene Hansen[1], Nina Milora[2], Giuseppe Copani[2]

[1] University of Copenhagen, Frederiksberg C 1870-Dk, Denmark
[2] Chr. Hansen Animal Health and Nutrition, Denmark

Direct-fed microbials (DFM) are used in ruminant production to increase productivity and improve health, they can be used as a possible option to decrease methane emission. The objectives of this study was i) to validate the same test in two laboratories and ii) to asses the effect of two DFM products in an *in vitro* rumen fermentation. Six treatments (n=3/treatment) were fermentated twice for 48 hours using an automated gas pressure measurement system, simultaneously at University of Copenhagen and Chr. Hansen. Maize silage (MS) and grass silage (GS) was fermented with and without two DFM products (P1: *Lactobacillus animalis, Propionibacterium freudenreichii* $1.5*10^7$ total CFU/ml, P2: P1 + *Bacillus subtilis*, *Bacillus licheniformis* $5.9*10^7$ total CFU/ml). Rumen fluid was added to a buffer solution (2:1) and kept anaerobic at 39.5°C. The test units were kept in a thermoshaker with 40 rotations per minute for 48h. Total gas production (TGP: ml at Standard Temperature and Pressure/gram organic matter), pH, organic matter degradability (dOM), methane concentration (MC) and volatile fatty acids (VFA) were measured after fermentation. All the measured parameters were blank corrected before statistical analysis. No significant effect of laboratory was detected for any of the response variables. Therefore, a mixed linear model with laboratory as a random variable and feed and additive was used. Significance was determined if $p<0.05$. The dOM of MS (78.3%) was significantly different from GS (81.4%) regardless of the additives (P1 and P2), however there were no significant differences of the effect of either probiotic within the feed type. Degradation of MS produced significantly more gas than GS after 48 hours. Both probiotics increased TGP significantly in GS but not in MS. There was no difference in total VFA production, however GS with and without probiotics produced signifincantly more propionic acid and less butyric acid then MS with and without probiotics. The addition P2 numerically reduced the total methane production per gOM between 4-6% in both MS and GS. These results showed that i) there is alignment between the two tested laboratories and ii) the P2 DFM shows potential to reduce methane production without affecting organic matter degradation.

[P02] HIGH-THROUGHPUT PHENOTYPING OF 239 LEUCONOSTOC STRAINS

Vera Kuzina Poulsen[1], Lucia Herrera-Dominguez[1], Elke Brockmann[1], Gunnar Oeregaard[1]

[1] Chr Hansen, Hørsholm, Denmark

Leuconostoc can preserve and enhance the shelf-life, flavor, texture, and functional properties of fermented food. We have performed a high-throughput characterization of 239 *Leuconostoc* strains for their ability to grow with various sugars, milk acidification properties, production of volatile organic compounds, and texturing properties. The addition of sucrose enhanced the milk acidification speed and resulted in production of slimy colonies on agar typically attributed to homo-polysaccharide production by 38 % of the strains. Associations between the genetic content of the strains and their abilities to contribute to texturing properties and flavor of fermented products were made.

[P04] BIOACTIVITY AND SENSORY PERSPECTIVE OF PROBIOTIC YOGURT FORTIFIED WITH APPLE POMACE FLOUR

Marina Jovanović[1], Snežana Zlatanović[2], Marija Petrović[2], Jovanka Laličić Petronijević[3], Dragana Mitić-Ćulafić[1], Jelena Miočinović[3], Stanislava Gorjanović[2]

[1] University of Belgrade-Faculty of Biology, Belgrade, Serbia
[2] Institute of General and Physical Chemistry
[3] University of Belgrade - Faculty of Agriculture

Yogurt is one of the most popular functional dairy product commercially used as a carrier for gut-friendly probiotics and bioactive food ingredients. In recent years there has been a growing interest in apple pomace (AP) bioactivity and its efficacy as a health promoting food component. However, commercially available food with declared AP in its composition is still rare. Thus, to meet the demand of new functional foods, in line with the trend of sustainable development, in this study innovative technological process, dehydration bellow 55^{o}C and grinding without heating, was conducted to obtain apple pomace flour (APF) and a novel fermented dairy product with probiotics (i.e. probiotic yogurt) enriched with APF has been design and examined.

APF was obtained from mixed apple varieties: Idared, Jonagold, Golden Delicious and Granny Smith. Yogurts were prepared from pasteurized cow milk and lyophilized starter culture: *Lactobacillus acidophilus, Streptococcus thermophilus* and *Bifidobacterium* sp.. Unsweetened yogurt samples were fortified with APF in the concentration of 1%, 3%, and 5%. Viable bacterial counts were estimated by the spread plate technique. Cytotoxic effect of APF yogurts on human colon cancer cell lines HCT116 and SW-620 was evaluated with MTT assay, whilst the total phenol content and antioxidant properties were investigated by TPC, DPPH and FRAP assays. Textural properties of yogurts, firmness, consistency, cohesiveness and index of viscosity, were analyzed through a single compression test. The overall sensory quality was determined using the scoring method (0–5) whereby the representative sensory properties were evaluated: color, texture, flavor, odor and taste.

The bioactivity of yogurt was notably increased by addition of APF. Supplementation of yogurt with APF affected viability of probiotic strains, increased antioxidant properties and cytotoxic activity against both cancer cell lines. Probiotic yogurt with 5% APF showed highest total phenolic content, DPPH radical scavenging activity and inhibitory effect on HCT116 cells growth, whereas for the one with 3% APF the highest improvement in texture properties was detected and the highest sensory attributes score was assigned. Overall, APF demonstrated promising potential to be used as a fortifying ingredient in the development of novel yogurt with functional properties.

[P05] PRE-PRO-POSTBIOTICS AND ROLE IN MODULATING OUR MICROBIOME - STATUS & CASE STUDIES

Radhika Bongoni[1], Derek Butler[1]

[1] Baseclear Bv, Leiden, Netherlands

Microbiome makes us complete. To feed, support and maintain a balance in microbial community is thus pivotal. Therefore the use of pre, pro and post (and many other versions) of biotics have been taking a key role in the industry for human, animal including aqua and plant health. Thus several companies are offering products with specific application targeting a health segment. Consumers should be informed on the scientific backing of such products.

In skin care -dermatological applications, pre and post biotics mostly via atopic application have shown positive evidences. While for, gut health its a combination of pre-probiotics, depending on the condition of the health, that it showed any significant positive effect. However, interesting results are for the use of these biotics during 'window of opportunity' from pregnancy to infancy to first 2 years of life, that can manoeuvre gut but also conditions like proneness to atopic dermatitis. This presentation is a snapshot of scientific evidences put forth as case studies for the above applications. Until now the applications of these biotics has been on disease management rather disease cure. This presentation further takes you through the state-of-art genomics technologies that might aid the quest in understanding the role-mechanism of pre-pro-postbiotics for (human)health.

[P06] THE EFFECT OF PECTINS ON ADHESION OF LIMOSILACTOBACILLUS FERMENTUM PCC AND INTEGRITY OF THE CACO-2 CELL MONOLAYER

Thanyaporn Srimahaeak[1], Fernanda Bianchi[2], Nadja Larsen[1], Lene Jespersen[1]

[1] *University of Copenhagen, Frederiksberg, Denmark*
[2] *Department of Food Science, Unesp - São Paulo State University, Araraquara, Brazil*

Pectins are complex plant polysaccharides used in food industry as stabilizers and gelling agents. They are commonly referred as emerging prebiotics, which beneficial effects for human health still need to be confirmed. Recent studies indicate that growth and adhesion of probiotic bacteria in the gut can be enhanced by prebiotic fibres. This study investigated the effect of four citrus pectins on adhesion of the probiotic *Limosilactobacillus fermentum* PCC to human colorectal adenocarcinoma cells Caco-2 and integrity of the intestinal epithelial monolayers using the Caco-2 cell model. Adhesion of *L. fermentum* PCC was enhanced (from 35% to 54–73%) in the presence of pectins with high and moderate degree of esterification (DE), whereas it was reduced (to 2%) by a pectin with low DE. Transepithelial electrical resistance (TEER) was significantly increased upon exposure to *L. fermentum* PCC alone (by 33%) and, to a greater extent, by combinations with pectins (by 44 – 48%), suggesting synergistic action of bacteria and pectins. Increases in TEER were related to transcriptional responses of the tight junction genes, encoding claudin-4 and claudin-2 proteins. The overall results indicated that pectins, when applied in synbiotic combination with *L. fermentum* PCC, have potential to improve bacterial adhesion and the intestinal epithelial barrier.

[P07] CRAFTING FERMENTATION NUTRIENTS IS KEY FOR DEVELOPING EFFICIENT & INDUSTRY-READY CULTURE MEDIA FOR PROBIOTICS & MICROBIOME-BASED STRAINS MANUFACTURING

Micheline EL Khoury[1], Benoît Drogue, Chloé Navas and Alain M. Sourabié

[1] Lesaffre, France, USA, China & Singapore

There has been an increasing interest in the recent decades for incorporating probiotic bacteria into different food and in developing formulations for nutraceutical and microbiome related applications. As a result, substantial efforts are undertaken by manufacturers on searching for technologies to enhance probiotic strains culture yield and to maintain functionality during the bioprocess and ultimately in the GIT. There are many technological challenges in producing probiotics and Live Biotherapeutics products (LBP). From a manufacturer perspective, key targets include optimizing fermentation yields while preserving cell viability & vitality throughout downstream processes and in finished formulated product. This includes cells resistance to freeze drying, stability over time and even more in anaerobic conditions. Individual cell lines have unique and complex nutritional requirements which are not fully covered using commercial culture media. Furthermore, the composition of industrial culture media for probiotics and LBP production is constrained by the associated COGS.

This paper presents Procelys efforts to design custom media formulations allowing to maximize commercial production yields. The primary objective was to develop fit-for-purpose, cost-effective, non-animal and non-allergenic yeast-based nutrients (YBN), extracts and peptones for probiotic and LBP strains e.g., Lactobacilli, Bifidobacteria and Faecalibacterium prausnitzii industrial manufacturing. Our findings suggest the specific growth rate of most strains, their cells morphology and physiological traits such as viability and ability to stay stable during the shelf life were significantly influenced by YBN type and concentration. Moreover, we demonstrated cells resistance to gut acidity and bile as well as their efficacy in inflammatory response reduction as well as on leaky gut diminution were highly impacted by their growth conditions and mainly the YBN type utilized in the respective culture media.

The current study showcasing the long-term expertise of Procelys Applications Labs and Technical Support will assist the probiotics and LBP industries for the careful selection of optimal fermentation nutrients. The NuCel® and ProCel® YBN range especially designed by Procelys are the state-of-the-art solutions to enable robust cell growth and high stability over time for probiotics and LBP strains, in addition of being fully compliant respectively with Food and Pharma regulations.

SESSION: YEAST

[P08] IN VITRO STUDIES OF THE EFFECTS OF SACCHAROMYCES CEREVISIAE ON EPITHELIAL BARRIER INTEGRITY COMPROMISED BY CANDIDA ALBICANS 3135

Thanyaporn Srimahaeak[1], Liesbeth Demuyser[2], Nadja Larsen[1], Lene Jespersen[1]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] Center for Microbiology, Vib Ku-Leuven, Belgium

Introduction
Candida albicans is able to invade mucosal surfaces leading to epithelial cell. There is scientific evidence that some strains of *Saccharomyces cerevisiae* have the ability to prevent cell disruption caused by pathogenic microorganisms. The aim of this study was to investigate the ability of *S. cerevisiae* to protect epithelial barrier integrity against *C. albicans* infection *in vitro*.

Methods
The study included three nonpathogenic strains of *S. cerevisiae* strains (SbP, D7 and I-3856) and a clinical isolate of *C. albicans* 3135. Integrity of the intestinal epithelial cell barriers upon exposure to yeasts was evaluated by measurements of transepithelial electrical resistance (TER) across the human colon adenocarcinoma cells Caco-2 for 24 hours. In setup 1, the Caco-2 monolayers were pre-incubated with *S. cerevisiae* for 5 hours followed by loading either *C. albicans* or *S. cerevisiae* cells. For setup 2, the pre-incubation step was performed with the cell culture medium before challenging with *C. albicans*. Morphological changes in *C. albicans* cells as affected by *S. cerevisiae* were observed by crystal violet staining and inverted microscopy.

Results
Challenging of the Caco-2 monolayers with *C. albicans* 3135 resulted in disruption of the Caco-2 monolayers already after 12 hours of treatment (setup 2). In contrast, the TER values were increased upon exposure to individual strains of *S. cerevisiae* (setup 1) throughout 24 hours incubation, indicating their potential to strengthen intestinal barrier integrity. Pre-treatment with *S. cerevisiae* SbP, D7 and I-3856 led to a delay in disruption of the monolayers by *C. albicans*, among them, the highest protective effect was observed for *S. cerevisiae* D7. All tested strains of *S. cerevisiae* reduced hyphal formation of *C. albicans* applied to the Caco-2 cell monolayers.

Conclusion
S. cerevisiae has a potential to improve intestinal barrier functions and prevent hyphal development in *C. albicans*. The mechanisms behind yeast interactions need to be further elucidated.

[P09] TASTING THE TERROIR OF WINE YEAST INNOVATION

Sakkie Pretorius[1]

[1] Macquarie University, Sydney, Australia

Wine is an archetypal traditional fermented beverage with strong territorial and socio-cultural connotations. Its 7,000-year history is patterned by a *tradition* of *innovation*. Every value-adding innovation that led to the *invention* of a new *tradition* spurred progress. It should not be assumed *a priori* that tradition and innovation are polar opposites. Innovation can strengthen wine tradition, and the reinvention of a tradition-bound practice, approach or concept can foster innovation. Therefore, finding the right balance between *traditions worth keeping* and *innovations worth implementing* can be complex. The intent here is to harness the creative tension between *science fiction* and *science fact* when innovation's first-principles challenge the *status quo* by re-examining the foundational principles about a core traditional concept, such as *terroir*. Poignant questions are raised about the importance of the *terroir* (biogeography) of yeasts and the value of the microbiome of grapes to wine quality. This presentation imagines a metaphorical *terroir* free from cognitive biases where diverse perspectives can converge to uncork the effervescent power of territorial yeast populations as well as 'nomadic' yeast starter cultures. At the same time, this paper also engages in *mental time-travel*. A future scenario is imagined, explored, tested and debated where *terroir*-less *yeast avatars* are equipped with designer genomes to safely and consistently produce, individually or in combination with region-specific wild yeasts and or other starter cultures, high-quality wine according to the preferences of consumers in a range of markets. The purpose of this review is to look beyond the horizon and to synthesise a link between *what we know now* and *what could be*. This presentation informs stakeholders where to *look* without suggesting what they must *see* as a way forward. In the context of one of the world's oldest fermentation industries, the mantra here is: *respect the past*, *lead the present*, and *secure the future* of wine.

[P10] INFLUENCE OF NITROGEN SOURCE ON EXPRESSION OF GENES INVOLVED IN AROMA PRODUCTION IN SACCHAROMYCES UVARUM

Angela Coral Medina[1], Carole Camarasa[2], John Morrissey[3]

[1] University College Cork - Supagro Universite de Montpellier, Cork, Ireland
[2] Inra, Supagro, Universite de Montpellier
[3] Ucc, Cork, Ireland

Saccharomyces uvarum has interesting properties that can be exploited for the production of fermented beverages. Particularly, the cryotolerance and capacity to produce high amounts of volatile compounds offers new opportunities for the fermentation industry. Besides the contribution of the nitrogen source to primary metabolism, some nitrogen compounds are precursors of volatile molecules that produce aroma. The nitrogen compounds assimilated by yeast are classified as rich or poor nitrogen sources depending on how they affect the specific growth rate. In *S. cerevisiae*, the nitrogen metabolism is well understood but less is known about these pathways in *S. uvarum*, nor whether there are regulatory differences between the two yeasts. We explored the nitrogen metabolism in *S. uvarum* and the nitrogen source effect on the production of aroma volatiles at low temperature. The focus is on temperatures below 20°C since this is relevant for production of wine or cider. First, nitrogen preference was established using 10 different compounds as sole nitrogen sources for *S. uvarum* and *S. cerevisiae*: important differences were found in the efficiency of asparagine to support growth. Afterwards, comparative analysis of gene expression (RNAseq) of *S. uvarum MTF3098* was carried out in ammonium, methionine, phenylalanine and asparagine to determine how the nitrogen source affects the expression of key genes involved in nitrogen metabolism and aroma production. The transcriptome data revealed substantial changes in expression patterns of genes encoding transporters and proteins responsible for aroma synthesis; using amino acids as sole nitrogen source instead of ammonium resulted in an increased expression of this group of genes. Furthermore, the gene expression results were related to the volatile metabolome analized in *S. uvarum* and *S. cerevisiae* in wine conditions. This study increases understanding of the importance of the nitrogen source in the aroma production of *Saccharomyces* yeasts and broads the knowledge on *S. uvarum* aroma production for applications in wine and cider industry.

[P11] STRAIN DIVERSITY IN VITAMIN B1 YIELD IN SACCHAROMYCES CEREVISIAE

Rebecca Rocchi[1], Eddy J. Smid[1]

[1] Wageningen University, Wageningen, Netherlands

Yeasts are highly exploited for the production of flavours. Yeast biomass itself is valuable in food applications by being converted to yeast extracts, which are used for their umami flavour as an alternative to glutamate[1]. Yeasts can also produce numerous vitamins. *S. cerevisiae* produces thiamine (vitamin B1). As awareness over health is gaining more attention, it would be interesting to increase thiamine levels in yeast extracts to use as flavour in (fermented) meat analogues, among other possible substrates. In this study the capability of different strains of *S. cerevisiae* to produce vitamin B1 is investigated. A screening of different yeast strains, isolated from various food sources, was carried out in Wickerham minimal medium lacking amino acids and thiamine. In this screening intracellular and extracellular thiamine production was measured among the different strains. The screening showed large variation in thiamine production and excretion among the tested strains, ranging in the ppm levels for intracellular thiamine content and ppb for extracellular. Intracellular thiamine levels are shown to be growth stage dependent.

1)Aygul Alim and others, 'The Behavior of Umami Components in Thermally Treated Yeast Extract', Food Research International, 120.August 2018 (2019), 534–43 <https://doi.org/10.1016/j.foodres.2018.11.002>.

SESSION: WHOLE GENOME SEQUENCING

[P12] OPTIMAL CONDITIONS FOR VIROME SAMPLE PRESERVATION REVEALED BY BACTERIOPHAGE INFECTIVITY AND VIROME METAGENOMIC ANALYSIS

Xichuan Zhai[1], Antonios Aslampaloglou[2], Josue L. Castro Mejia[3], Dennis Sandris Nielsen[3], Ling Deng[4]

[1] Section for Food Microbiology and Fermentation, Department of Food Science, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark, Copenhagen, Denmark
[2] Aalborg, København, Denmark
[3] University of Copenhagen, Frederiksberg, Denmark
[4] Section of Food Microbiology and Fermentation, Department of Food Science, Facuty of Science, University of Copenhagen, Denmark

Virus is an essential part in the gut microbiota, and the study of gut virome has emerged as a hot topic. However, there is no systematic study has evaluated the effect of storage conditions (buffers, temperatures, and time) on the phages infectivity and the viral metagenomes of fecal virome samples. In the present study, we longitudinally evaluated 5 different preservation buffers (StayRNA, CANVAX, DNA/RNA Shield, RNAlater, and SM buffer) on the infectivity of spiked phages T4, c2, and Phi X174 and the fecal viromes with combinations of different temperatures and storage time. Our results demonstrated that the infectivity recovery rate of the different bacteriophages is variable and highly dependent on storage buffers. We also found longer time storage (500 days) at – 80°C affect the viral diversity in the different buffers. SM buffer stored virome showed a 10~20% increase in the relative abundance of Caudoviales while the other buffers increased the "dark matter of virome" that cannot be assigned. It also appeared that the samples stored in CANVAX or DNA/RNA Shield buffer stored at -80°C has the least shifts in metagenomics analysis during the longer time storage. Our study provides a comprehensive view to the impact of storage conditions on fecal viral activity and genome. Based on the results, we recommend SM buffer at 4°C for virome samples that can be treated within 2 weeks, and CANVEX or DNA/RNA Shield for longer storage at -80°C.

Keywords: Phage, Virus, Storage, Buffer, Sequencing

SESSION: ENZYMES FOR FOOD AND FEED APPLICATIONS

[P13] EXTRACELLULAR PRODUCTION OF POLYSACCHARIDE-DEGRADING-ENZYMES BY MUCOR SP. THROUGH FERMENTATION OF BREWERS SPENT GRAIN

Shady Hassan[1], Brijesh K. Tiwari[2], Gwilym A. Williams[3], and Amit K. Jaiswal[4]

[1] Technological University Dublin, Dublin, Ireland

[2] Department of Food Biosciences, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

[3] School of Biological and Health Sciences, College of Sciences and Health, Technological University Dublin, Kevin Street, Dublin, Republic of Ireland

[4] School of Food Science and Environmental Health, College of Sciences and Health, Technological University Dublin, Cathal Brugha Street, Dublin, Republic of Ireland.

The potential of microwave and ultrasound was evaluated for the pretreatment of brewer's spent grain (BSG). The highest sugar yield was obtained (64.4±7 mg) when 1 g of BSG was pretreated with microwave energy of 600 w for 90 s. Afterwards, the pretreated BSG was evaluated as a substrate for production of Xylanopectinolytic enzymes using fungi isolated from spoiled fruits. Out of twenty-nine (29) isolates recovered, *Mucor sp*. (AB1) isolated from Bramley apple (*Malus domestica*) produced xylanopectinolytic enzymes with higher specific activity, and were selected for further studies. The highest enzyme activity (123 U/g, and 67 U/g BSG, for pectinase and xylanase, respectively) was achieved in a medium that contained 15 g of BSG, at pH 6, temperature of 30°C, and supplemented with 1 % xylan or pectin for inducing the production of xylanase or pectinase, respectively.

Keywords: Lignocellulose; Brewer's spent grain; Microwaves; Pectinase, Xylanase.

[P14] A MULTI-ENZYME CARBOHYDRASE PRODUCT IMPROVES FIBER SOLUBILIZATION OF CEREALS AND OIL SEEDS

Eduardo Antonio Della Pia[1], Morten Broch Tovborg[1], Philippos Fidiarakis[2]

[1] Novozymes A/S, Lyngby, Denmark
[2] Dsm Nutritional Products AG, Kaiseraugst, Switzerland

Carbohydrases can hydrolyze non-starch polysaccharides (NSP) into oligomers, release nutrients encapsulated by the cell walls, and reduce the viscosity of the animal digesta. MultiGrain® (DSM Nutritional Products, Basel, Switzerland) is a carbohydrase multi-enzyme product expressed by *Trichoderma reesei* that is used as feed additive in animal nutrition. This microorganism has been previously recognized as an exceptional producer of fiber-degrading enzymes that can synergistically hydrolyze complex cell wall structures.

By using state-of-the-art proteomic technology, we investigated the protein composition of this multi-enzyme product. The proteome analysis was performed by using a Thermo Orbitrap Velos Pro LC/MS instrument and the enzyme proteome of the *T. reesei* fermentation product was compared to the *in silico* predicted corresponding *T. reesei* secretome. The secretome was predicted from the *T. reesei* genome sequence using the SignalP 4.1 software solution. The LC/MS analysis gave new insights into the plant polysaccharide enzymatic degradation potential of the *T. reesei* product. The analysis demonstrated that MultiGrain® contains a multitude of enzymatic activities targeting hemicellulose, glucans, and protein. Among many others, we identified different endo-xylanases, β-glucanases, xyloglucanase, and several cellulases and debranching enzymes. As all these activities are important in degrading complex carbohydrates (cellulose, hemicellulose, arabinoxylan, β-glucans, xyloglucan, …) found in feed materials, we tested the *in vitro* efficacy of the multi-enzymes cocktail in hydrolyzing fibers of both cereals such as wheat, barley, rye, oat, rice and protein-rich seeds such as canola and sunflower. By using a combination of *in vitro* incubations, oligo and monosaccharide analysis, viscosity measurements and confocal microscopy we have shown that complex NSP polymers of all the different substrates can be solubilized by the action of the enzyme product.

The results summarized in this paper provide strong evidence that Multigrain® can help releasing nutrients from their encapsulation in cereal cell wall structures by the synergistic effect of many different enzymatic activities and thereby (i) improve utilization of dietary nutrients, (ii) have a positive effect on livestock performance and (iii) decrease the feed cost in poultry production.

[P15] MURAMIDASE INDUCED PEPTIDOGLYCAN DEPOLYMERIZATION IN VIVO IS MEASURED BY NOVEL LCMS-MS METHOD QUANTIFYING THE CONCENTRATION OF SOLUBLE- AND TOTAL MURAMIC ACID IN DIGESTA

Mads Pedersen[1], Carsten Østergaard Frederiksen[1], Estefania Pérez Calvo[2], Rual Lopez-Ulibarri[2], Mikkel Klausen[1]

[1] Novozymes A/S, Bagsværd, Denmark
[2] Dsm Nutritional Products

Generally, enzymes applied in animal feed formulations are all recognized to deliver performance by degrading or depolymerizing components in the ingested feed matrix e.g. to improve nutrient digestibility. Limited attention has been placed on identifying novel enzyme categories capable of improving animal performance, by targeting other components present in the gastro intestinal tract than those originating from the ingested feed. The gastro intestinal microbiota exists in a complex ecosystem in equilibrium with the host. Microbial cell division and death occur naturally throughout the entire gastrointestinal tract, resulting in a diversified release of defragmented microbial cell components into the gut lumen. The structural polymer peptidoglycan, exclusively found in bacterial cell walls, comprises a significant fraction of the cell wall in all bacterial species present in the gastrointestinal tract. Recent studies reported how *in vivo* supplementation of a novel fungal muramidase enzyme, capable of depolymerizing peptidoglycan from dead bacteria, significantly improved performance parameters in broiler chickens. To further investigate the muramidase impact on peptidoglycan depolymerization *in vivo*, a LCMS-MS method was developed to quantify the concentration of total and soluble muramic acid after acid hydrolysis in digesta samples. The total concentration of muramic acid in digesta increased through the gastro intestinal tract with the highest concentration observed in caecum. Interestingly, when examining the ratio between the soluble and total muramic acid concentration, a significant increase was observed by muramidase supplementation (45.000 LSU/kg) in all analyzed segments; crop, jenunum and caecum ($P<0.05$). The effect of muramidase supplementation was most pronounced in jejunum with approximately twice as high soluble-to-total muramic acid ratio compared to the control. In addition, a significant effect of muramidase dosage on the soluble-to-total muramic acid ratio was observed when supplementing three different dosages of muramidase (25.000, 35.000, and 45.000 LSU/kg) in ileum collected samples ($P<0.05$), emphasizing the small intestine as a key segment associated with muramidase performance and effect *in vivo*.

[P16] CELL SURFACE ATTACHMENT OF THE LACTOCOCCUS LACTIS PRTP PROTEASE

Ida Nynne Laforce[1], Egon Bech Hansen[1]

[1] Technical University of Denmark, Lyngby, Denmark

Lactic acid bacteria (LAB) adapted to nutrient rich environments are typically auxotrophic for several amino acids and depend on external supply of peptides and amino acids. Milk does not contain sufficient low molecular weight nitrogen to allow lactic acid bacteria (LAB) to grow to high densities in the absence of proteolysis.

Proteolytic strains of *Lactococcus lactis* used in dairy fermentations carry cell envelope proteinases (CEP) attached to the surface of the cell. The enzyme, PrtP, is with more than 1900 amino acids a very large enzyme. All proteolytic isolates of milk associated *L. lactis* strains have a PrtP proteinase with an anchor domain allowing sortases to attach the enzyme covalently to the outer surface. The covalent attachment of the CEP seems to be a stable property in *L. lactis*, and we would assume covalent attachment to confer a clear advantage.

We have studied the phenotype of covalent attachment of CEP in *L. lactis* by constructing a set of isogenic strains carrying two versions of PrtP enzymes. One version is the wild type version with the enzyme covalently attached to the cell wall and the other version is a truncated enzyme with a deletion of the W, H, and Anchor domains. We used six different PrtP enzymes (NCDO712, Wg2, HP, SK11, MS22333, and MS22337). The *L. lactis* strain MG1363 was used as the host.

All 12 constructs grow surprisingly well in pasteurized and homogenized bovine skim milk. Covalent attachment seems to make very little difference in this milk; all 12 strains acidify fast and deeply. The three domains H, W and Anchor seems to be dispensable in ordinary bovine skim milk.

However, in raw milk we observe differences and strains behave different depending on the species of the milk being camel or cow.

Cell surface attachment seems in raw milk to be beneficial during the growth transition when low molecular weight nitrogen is exhausted and the proteolysis becomes essential. Cells with a covalently attached protease are in raw camel milk coping better with this transition in comparison to cells expressing a secreted PrtP

[P17] WATER KEFIR REPRESENTS A RESERVOIR FOR LACTIC ACID BACTERIA ENCODING NOVEL GLUCANSUCRASES

Julia Bechtner[1], Frank Jakob[1], Rudi F. Vogel[1]

[1] Technische Universität München, Freising, Germany

Water kefir is a traditional beverage prepared from water, dried fruits, lemon slices and sucrose. The fermentation is accomplished by a predictively symbiotic multi-species consortium including yeasts and bacteria, i.a. lactic acid bacteria (LAB) that reside in or on a polysaccharide biofilm matrix – the kefir granules. These granules are of gelatinous consistency and mostly subside to the bottom during fermentation, while the upper aqueous phase becomes turbid over time. While it has been proposed that *Lentilactobacillus hilgardii* is the main producer of the water-insoluble granule polysaccharide, it is unknown so far how other LAB contribute to the formation of this beverage. Therefore, we investigated water kefir borne strains of the genera *Liquorilactobacillus, Lentilactobacillus* and *Leuconostoc* for their capability of producing α-glucan from sucrose. Subsequently, whole genome sequences were obtained for a selection of these strains and searched for the presence of glucansucrase-encoding genes. Herein, it could be shown that *Leuconostoc* strains encoded at least two glucansucrases with a high intra- and interspecies diversity, while *Liquorilactobacillus* and *Lentilactobacillus* strains predominantly encoded only one of these enzymes, which were highly similar within one species. Including well-characterized enzymes, this sequence comparison furthermore suggested the novelty of several of the glucansucrases identified within water kefir LAB. A detailed investigation of the novel glucansucrases from *Liquorilactobacillus hordei* and *nagelii* revealed both microorganisms to produce dextran-type glucans, which is responsible for the stable turbidity of the beverage. Our results thus showed that glucan formation in water kefir is functionally divers and extends much beyond granule formation. The identification of a variety of different novel glucansucrases furthermore proofs water kefir to be a yet unexploited reservoir for LAB with biotechnological application potential, namely innovative food fermentations.

SESSION: MICROBIALLY DERIVED BIOACTIVE COMPOUNDS

[P18] MALT FLAVOUR IN SWISS SEMI-HARD CHEESE

Meng Yi Hélène[1], Baumeyer Brahier Alexandra[1], Tena Stern Mireille[1], Dubois Sébastien[1], Schmidt Remo S.[1], Fuchsmann Pascal[1], Ueli von Ah[1]

[1] *Agroscope, Bern, Switzerland*

Flavour perception is one of the key factors in determining the preference of a consumer for a food product, implying that an overall balance of the aroma compounds is important. In some Swiss cheeses and also other products such as e. g. sourdough bread, malt aroma is desirable. In the present study, however, we worked with a cheese type which is characterised by its buttery, fruity, herbal notes and its ability to melt. Therefore, maltiness was considered as an off-flavour.

Using whole genome analysis and model cheese experiments we aimed to understand the influence of the starter culture composition on the formation of the compounds responsible for malt flavour. In order to link these geno- and phenotypes, we took advantage of machine learning algorithms, allowing us to further understand the strain interactions and a possible prediction of the *in vitro* outcome *in silico*.

Five compounds were previously identified by dynamic headspace-vacuum transfer in trap-gas chromatography-mass spectrometry-olfactometry (DHS-VTT-GC-MS-O) as the main responsible for the malt aroma: 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, 2-methylbutanol and 3-methylbutanol. In a series of experiments using small model cheeses, we determined the concentration of a selection of these compounds at different time points after spiking with their respective precursor compounds. With these measurements, we then trained random forest models using the observed compound concentrations as the response and the underlying genomic information as the features, allowing us to study the importance of each genetic feature and to predict an outcome with new data.

With our technique to study malt aroma formation, it may be possible to predict the formation or degradation of 3-methylbutanal in a mixture based on the genome data of the single strains and further confirm the presence/absence of 3-methylbutanal with a simple small scale cheese model. The same approach could also be used for other products such as sourdough bread, spirits and beers, to name a few.

[P19] HOW TO SCREEN FOR AGENTS THAT PREVENT LATE BLOWING DEFECT IN CHEESE

Sofia Dashko[1], Hugo Streekstra[1], Sijin Liu[1], Paul Klaassen[1]

[1] *Dsm Food Specialties B.V., Delft, Netherlands*

A screening system to test inhibitory substances and cultures against gas-producing *Clostridium tyrobutyricum* was developed. Four model systems were compared: Reinforced Clostridial Medium (RCM), Enriched Milk (EM), cheese slurry and miniature cheese. Each of these systems had their specific pros and cons, no single system being able to accommodate both the testing of inhibitory substances and inhibitory cultures. For anti-clostridial substances, RCM and cheese slurry are recommended. RCM serves as a preliminary test model, with the cheese slurry serving as a good follow-up model under more application-relevant conditions. To test the protective cultures, a combination of miniature cheese and cheese slurry model is recommended, because these together offer insight in the freshly-made and late ripening period of cheese manufacture.

[P20] LACTOCOCCAL EXTRACELLULAR MEMBRANE VESICLES DELIVER BIOACTIVE VITAMIN K2 TO HUMAN CELLS

Yue Liu[1], Eline van Ophem[1], Eddy J. Smid[1], Tjakko Abee[1]

[1] *Wageningen University, Wageningen, Netherlands*

Vitamin K2 (menaquinone, MK-n) is a lipophilic vitamin located in bacterial cell membranes and essential for human health as a carboxylation co-factor. The long-chain forms of vitamin K2 show a better retention in the serum and have a better bioavailability for target tissues in the human body compared to the short-chain forms. However, the strong lipophilicity of long-chain vitamin K2 forms poses challenges to their uptake by target cells of the human host to achieve desired biological function. In this study, bacterial extracellular membrane vesicles (EVs) produced by *Lactococcus lactis* were shown to carry mainly long-chain vitamin K2 (MK-8 and MK-9). When these EVs were applied to *in vitro* grown osteosarcoma cells, the ratio of carboxylated over non-carboxylated osteocalcin increased, indicating functional delivery of bioactive vitamin K2 by bacterial EVs. The efficiency of vitamin K2 delivery by EVs was higher than adding solvent-dissolved pure compounds at similar concentrations. Therefore, this study provides proof of principle that bacterial EVs are ideal vehicles to deliver lipophilic compounds like vitamin K2 to human cells. Investigation on EVs produced by bacteria with GRAS status that are key players in food fermentations, will promote the applications of bacterial EVs in efficient delivery of bioactive, nutritional compounds from the microbial origins to the human host, contributing to improved human nutrition and conceivable health benefits.

[P21] EFFECT OF A DUAL-PURPOSE SILAGE INOCULANT ON PATHOGENIC ESCHERICHIA COLI O157:H7 IN GRASS SILAGE

Audrey Segura[1], Antonio Gallo[2], Nina Milora[3], Giuseppe Copani[3]

[1] *Chr. Hansen, Hoersholm, Denmark*
[2] *Department of Animal Science, Food and Nutrition (Diana), Università Cattolica del Sacro Cuore, Italy*
[3] *Chr. Hansen Animal Health and Nutrition, Denmark*

Spoiled and contaminated silages can be a source of pathogenic microorganisms that may impact animal performance, impair animal and human health, and constitute a vehicle of transmission of pathogens on the farm. Forages can be contaminated by *Escherichia coli* O157:H7 via manure, irrigation water, or poor farm management. The objective of this study was to evaluate the effect of a silage inoculant on silage fermentation quality, aerobic stability (AS), and the growth of *Enterobacteriaceae* including *E. coli* O157:H7 during fermentation and at the feed-out stage. Wilted grass was ensiled for one and two weeks and treated with: Water (Control, C); SiloSolve® FC (*Lactobacillus buchneri* DSM22501 and *Lactococcus lactis* DSM11037, applied at $1.5x10^5$ CFU/g) (FC); *E. coli* O157:H7 DSM17076 applied at 10^6 CFU/g (EC); and EC+FC. At opening, mini-silos followed an AS test for seven days. Each mini-silo was analyzed for fermentation end-products, yeast, mold, *Bacillus* spores, and *Enterobacteriaceae* at the end of the fermentation and after AS test. FC silages resulted in a faster pH decrease at both opening timepoints ($P<0.01$) and an increase in acetate ($P<0.0001$) and lactate ($P<0.05$). Furthermore, AS was improved by 125 hours after one week of fermentation ($P<0.0001$) and by 148 hours after two weeks of fermentation ($P<0.0001$). Overall, after two weeks of fermentation, FC silages had a significantly lower level of yeast (2.8 log CFU/g decrease, $P<0.05$), mold (4.1 log CFU/g decrease, $P<0.01$) and *Bacillus* spores (0.3 log CFU/g decrease, $P<0.01$). EC+FC silages reported lower *Enterobacteriaceae* compared to EC silages (2.7 log CFU/g decrease, $P\approx0.07$ after one week of fermentation and 7.3 log CFU/ml reduction, $P<0.01$ after AS test). Following two weeks of fermentation, *Enterobacteriaceae* survived in EC silages (3.2 log CFU/g) and were below the detection limit (<2 log CFU/ml) in EC+FC mini-silos. After AS test, *Enterobacteriaceae* were below the detection limit in all mini-silos. This study showed that SiloSolve® FC improves fermentation quality, extends AS, and reduces spoilage microorganisms after a short fermentation period. SiloSolve® FC can also be used to reduce the risk of silage contamination by pathogenic microorganisms, whereas poor management can increase the level of pathogenic *E. coli* O157:H7.

[P22] DIETARY FIBRES AND MUCINS AS MODULATORS OF GUT MICROBIOATA - IN VITRO STUDY

Grete Raba[1], Ana Sofia de Jesus Vaz Luis[2], Hannah Schneider[2], Indrek Morell[3], Signe Adamberg[4], Gunnar C. Hansson[2], Kaarel Adamberg[1], Liisa Arike[2]

[1] Tallinn University of Technology, Tallinn, Estonia
[2] Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden
[3] Center of Food and Fermentation Technologies, Tallinn, Estonia
[4] Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia

Dietary fibres (DFs) have gained a large popularity due to their potential as prebiotics. These complex polysaccharides are poorly digested by humans and pass through the intestinal tract where they come into contact with gut microbiota. The bacteria can harness their pool of specific enzymes to break down and utilise DFs as an energy source. Depending on the types of chemical bonds and/or physical properties such as solubility, different DFs have a varying effect on the development of bacterial consortia.

The fermentation of DFs results in metabolites which are beneficial to the host, especially short-chain fatty acids (SCFAs) which have been shown to have several health-promoting effects. One such benefit is the protection of the colonic epithelium. SCFAs are an energy source for the epithelial cells. Additional protection comes from the competition for living space. The epithelium is covered by a mucus layer, composed of mostly Mucin2 glycoprotein secreted by the goblet cells at the base of the colonic crypts. For the colonic health it is essential that the abundance of mucin-degrading bacteria is balanced by the fibre-degrading bacteria, so that the mucus layer could be renewed in time.

A panel of 14 DFs was studied for their gut microbiota modulating capabilities. The DFs were chosen to cover a range of physiochemical properties. Additional co-fermentations with mucins were done to elucidate the interplay between fibre-degrading and mucolytic species. Both the chemical and physical properties of the substrates were seen to affect the consortium development and the microbial metabolism. These results are the first part of a larger study of DF fermentation mechanisms.

[P23] ISOLATION OF COMMENSAL BACTERIA FROM PIGLET SMALL INTESTINE

Sami Aideh[1], Giuseppe Copani[2], Lukasz Krych[3], Tomasz Cieplak[1]

[1] Chr. Hansen, Horsholm, Denmark
[2] Chr. Hansen Animal Health and Nutrition, Denmark
[3] University of Copenhagen, Frederiksberg, Denmark

To elicit the positive effect on animal or human health, probiotic must pass through harsh environment of stomach (low pH) and duodenum (high bile concentration). Apart of that feed additive must pass jejunum and ileum. These parts of the gastrointestinal tract (GIT) harbors significant number of microbes that influence probiotic and feed behavior. So far none of the commercially available *in vitro* models simulate small intestine microbiota and to make the simulation closer to real life, small intestine simulated microbiota was created. The aim of this project was to isolate and characterize the most representative bacterial strains from the piglet small intestine and create an artificial stable small intestine consortium, that is representative of the *in vivo* conditions. This consortium will be further used in the *in vitro* GIT model. Total of 20 samples (from duodenum and from ileum) originating from two *in vivo* trials conducted by University of Copenhagen in Rødby and Grønhøj (Denmark) were collected (healthy animals). Bacteria were isolated anaerobically using 7 different agar culture media. The isolates were identified to strain level by ON-rep-seq, using Oxford Nanopore MinION. Further, the isolates were characterized for bile salts resistance and mixed in appropriate rate to mimic taxonomical composition of ileal microbiota. Initially over 250 strains were isolated, of which 192 were sequenced. Out of these isolates we identified 20 unique strains, belonging to 5 different genera. Bile resistance varied across samples and was highly dependent on bacteria taxonomy. By combing isolated strains, we were able to create a stable community, which highly resembled the by 16S rRNA gene-sequencing based characterization of the ileal microbiota. This small intestine consortium will be used in an *in vitro* GIT model that is currently under development and will help to understand the importance of upper intestine microbiota and probiotic mode-of-action. The use of the standardized consortium will also reduce the experiment to experiment variability and will be an useful tool for testing and compare feed additives under the same conditions. Above that using *in vitro* model with the consortium will allow us to reduce the use of animals for *in vivo* trials.

[P24] SELECTION OF PROBIOTIC STRAINS FOR MICROBIOME PRIMING IN PIGS

Lea Hübertz Birch Hansen[1]

[1] Chr. Hansen, Hørsholm, Denmark

Providing probiotics with the aim of establishing and maintaining a healthy gastrointestinal microbiota and thereby positively affecting animal health and performance is widely known. We hypothesize that supplying probiotics very early in the piglet's life increases the chance of beneficially affecting microbiota composition. When screening for potential probiotics for early inoculation of newborn piglets, it is paramount to decide upon which characteristics the probiotic strains should possess. The objective of this study was to identify probiotic strains with important functions for beneficially priming the gastrointestinal microbiota of the newborn piglet. Nine probiotic strains from the Chr. Hansen strain bank were selected based on stability and survival and other *in vitro* and *in vivo* results in animals and humans: *Bifidobacterium Longum* subsp. *Infantis, Bifidobacterium animalis* subsp. *Lactis, Bifidobacterium longum, Bifidobacterium breve, Lactobacillus rhamnosus, Lactobacillus acidophilus, Enterococcus faecium* and two *Bacillus subtilis* strains. The probiotics were assessed through an *in vitro* screening using four different assays: Growth on two porcine milk oligosaccharides (PMO), *E. coli* pathogen inhibition, Transepithelial/transendothelial electrical resistance (TEER) assay with *E. coli* challenge and competitive exclusion of *E. coli* through epithelial adhesion. Members of the genus *Bifidobacterium* were generally superior in growth on PMOs, however, not all members from this genus grew on the PMOs. *Lactobacillus acidophilus* also grew on one of the two PMOs. All nine probiotic strains inhibited growth of *E. coli* by creating inhibition zones around the probiotic spot. *Enterococcus faecium* was superior both in counteracting increased permeability in the TEER assay and competitively excluding adhesion of *E. coli* to epithelial cell lines. Members of the genus *Bacillus* did not show large effect in the two assays. The remaining strains were either effective in the TEER assay or in the competitive exclusion assay. Based on the *in vitro* screening, four probiotic strains (*Bifidobacterium Longum* subsp. *Infantis, Bifidobacterium breve, Lactobacillus rhamnosus and Enterococcus faecium*) with complementary characteristics were selected for an *in vivo* experiment testing early inoculation of selected probiotics to newborn piglets on robustness in weaned piglets being resistant to post-weaning diarrhea.

[P25] DEVELOPING METHODS FOR MICROBIOME ANALYSIS FOR ANIMAL HEALTH STUDIES

Ali May[1], Radhika Bongoni[1]

[1] Baseclear, Leiden, Netherlands

We are just beginning to understand the role of animal microbiome in animal health, nutrition, research, including solutions for greener agriculture. Veterinary researchers, feed manufacturers, animal breeders, and animal pharmaceutical companies are therefore looking for ways to characterise, if not alter, the animal microbiome for a wide range of applications. As a part of our mission in accelerating the understanding and use of microorganisms for a more sustainable, safer and healthier future, one of our goals at BaseClear is to become a leading partner in animal microbiome research. The projects we have worked on range from studying the relationship between diet and health in rhinos and monkeys, exploring the antibiotic resistance gene content in the chicken microbiome, to analysing the viral metagenome of fish to determine the viral cause of disease. To meet the niche requirement in each project, we continuously implement, optimise, and develop state-of-the-art techniques for sample collection, DNA/RNA isolation, preparation, sequencing, bioinformatics, and biostatistical analyses. In this meeting, we will share some of these efforts towards developing industry standards for animal microbiome analyses.

[P26] CHARACTERIZATION OF THE VAGINAL DNA VIROME IN HEALTH AND DYSBIOSIS: AN OPENING STUDY IN PATIENTS WITH NON-FEMALE FACTOR INFERTILITY

Rasmus Riemer Jakobsen[1], Thor Haahr[2], Peter Humaidan[3], Jorgen Skov Jensen[4], Witold Kot[5], Josue L. Castro Mejia[1], Ling Deng[6], Thomas Dyrmann Leser[7], Dennis Sandris Nielsen[8]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] Clinical Medicine, Aarhus University Hospital, Aarhus C, Denmark
[3] The Fertility Clinic Skive, Skive Regional Hospital and Department of Clinical Medicine Aarhus University, Skive, Denmark
[4] Statens Serum Institut, Copenhagen, Denmark
[5] Aarhus University, Roskilde, Denmark
[6] Section of Food Microbiology and Fermentation, Department of Food Science, Facuty of Science, University of Copenhagen, Denmark
[7] Chr. Hansen A/S
[8] Section of Microbiology and Fermentation, Dept. of Food Science, Faculty of Science, University of Copenhagen, Denmark

Background. Bacterial vaginosis (BV) is characterised by a reduction in *Lactobacillus* spp. abundance and increased abundance of facultative anaerobes, like *Gardnerella vaginalis*. BV aetiology is not fully understood, but bacteriophages could play a pivotal role causing perturbation of the vaginal bacterial community. Here we investigate the vaginal viral community, including bacteriophages, and its association to the bacterial community and BV-status.

Methods. Vaginal samples from 48 patients undergoing IVF treatment for non-female factor infertility were subjected to metagenomic sequencing of purified virus-like particles. The vaginal viral community was characterized and correlated with BV-status, bacterial community structure and presence of key vaginal bacterial species.

Results. The majority of identified vaginal viruses belonged to the class of double-stranded DNA bacteriophages, with eukaryotic viruses constituting 4% of total reads. Clear links between viral community composition and BV ($q = 0.006$, $R = 0.26$) as well as presence of *L. crispatus* ($q = 0.001$, $R = 0.43$), *L. iners, Gardnerella vaginalis* and *Atopobium vaginae* were found ($q < 0.002$, $R > 0.15$). Interestingly, also the eukaryotic viral community was correlated with BV-status ($q = 0.018$, $R = 0.20$).

Conclusions. The vaginal virome is clearly linked with bacterial community structure and BV-status.

[P27] IN VITRO IMMUNE MODULATORY EFFECT OF A THYMOL AND CINNAMALDEHYDE BLEND AND ORGANIC ACIDS

Darshana Kadekar[1], Katrine Jorgensen[2], Helene Pedersen[2], Karsten Kragh[3], Chong Shen[4]

[1] Iff Health & Biosciences, Brabrand, Denmark, Aarhus, Denmark
[2] Gut Immunology Lab, Iff Health & Biosciences, Brabrand, Denmark
[3] Iff Health & Biosciences, Brabrand, Denmark, Brabrand, Denmark
[4] Gut Immunology Lab, Iff Health & Biosciences, Brabrand, Denmark, Aarhus, Denmark

Introduction: After the ban on antibiotic growth promoters (AGPs), a range of dietary additives has been studied as alternatives, including organic acids and phytogenics. Organic acids (OA) have long been used as feed acidifiers, preservatives, as well as growth promoters and potential antibiotic substitutes. Our former studies have shown that a thymol and cinnamaldehyde blend (TCB) improves growth rate and digestibility, modulates the immune system and exerts anti-microbial effect. But only few studies have compared the effect of these additives.
Aim: The aim of this study was to assess the immune modulatory and anti-microbial effect of different OA and TCB.

Methods: Barrier integrity was investigated by permeability assay in pig epithelial cell line (IPEC-J2). TCB or OA pretreatment of IPEC-J2 cells was evaluated to examine the effect on the bacterial adhesion. Pig peripheral blood mononuclear cell (PBMC) culture was used to analyze cytokine expression by RT-PCR.

Results: Both TCB and OA improved the gut barrier function, as shown by 83% (TCB) and 60-70% (OA) less FITC-Dextran (4KD) leakage, respectively. Pretreatment of IPEC-J2 cells with TCB drastically reduced the attachment of pathogenic bacteria *(S. typhimurium, C. perfringens* and enteropathogenic *E. coli*). On the other hand, OA pretreatment showed 3-4-fold increased adhesion of the same pathogens. However, neither OA nor TCB significantly influenced the adhesion of *B. amyloliquefaciens* or LGG to the IPEC-J2 cells. Cytokine expression of PBMCs showed that TCB and OA have comparable anti-inflammatory effects as evidenced by downregulating pro-inflammatory cytokines IL-1β, IL-6 and TNF, while upregulating regulatory T cells transcription factor FoxP3.

Conclusion: Our data indicates that both TCB and OA enhance barrier function and exert anti-inflammatory effects. TCB but not all OA specifically prevent binding of pathogenic bacteria to gut epithelial cells, thus TCB has a better potential in feed additive application as AGPs replacement.

[P28] VITAMIN B12 AND BACTERIAL MICROCOMPARTMENTS STIMULATE THE ANAEROBIC GROWTH OF LISTERIA MONOCYTOGENES ON RHAMNOSE

Zhe Zeng[1]

[1] Wageningen University, Wagenigen, Netherlands

The food-borne pathogen *Listeria monocytogenes* can form proteinaceous organelles called bacterial microcompartments (BMCs) that optimize the utilization of substrates, such as 1,2-propanediol, and confer an anaerobic growth advantage. Rhamnose is a deoxyhexose sugar abundant in a range of environments including the human intestine, and can be degraded in anaerobic conditions into 1,2-propanediol, next to acetate and lactate. Rhamnose-derived 1,2-propanediol were found to link with BMCs in some human pathogens such as *Salmonella enterica*, but the involvement of BMCs in rhamnose metabolism and potential physiological effects on *L. monocytogenes* are still unknown. In this study, we firstly test the effect of rhamnose uptake and utilization on anaerobic growth of *L. monocytogenes* EGDe without and with added vitamin B12, followed by metabolic analysis. We unveil that the vitamin B12-dependent activation of *pdu* stimulates metabolism and anaerobic growth of *L. monocytogenes* EGDe on rhamnose via 1,2-propanediol degradation into 1-propanol and propionate. Transmission electron microscopy of *pdu*-induced cells shows that BMCs are formed and additional proteomics experiments confirm expression of *pdu* BMC shell proteins and enzymes. Finally, we discuss physiological effects and energy efficiency of *L. monocytogenes pdu* BMC-driven anaerobic rhamnose metabolism and impact on competitive fitness in environments such as the human intestine.

[P29] MAN'S BEST FRIEND: ANTIMICROBIAL COMPOUNDS ISOLATED FROM BACTERIAL STRAINS OF CANINE SOURCE.

Michelle O' Connor[1], Des Field[1], Colin Hill[1], Paul Ross[1]

[1] Department of Microbiology, University College Cork, Cork, Ireland

Antimicrobial resistance (AMR) is a major risk to human and animal health requiring urgent attention. A contributing factor to AMR is the misuse/overuse of antibiotics adding to the development and spread of resistance mechanisms by pathogens. Greater efforts are needed not only to prevent additional AMR development but to reassess and identify alternative therapeutic options. One group of compounds that hold great promise are antimicrobial peptides (AMPs).

AMPs produced by bacteria are termed bacteriocins, which can kill or inhibit bacterial strains closely-related or non-related to the producing strain. Notably, a diverse community of trillions of commensal bacteria inhabit the mucosal and epidermal surfaces of humans and animals and can be found in a multitude of sites; including the mouth, nose, skin, ears and intestines, many producing novel bacteriocins. Given their potent antimicrobial activity against pathogenic bacteria, immunomodulatory effects on their hosts, auto-regulation of their own production and self-immunity exhibited by the producing bacteria to their own peptides, this vast resource of bacteriocins could serve as promising alternatives to traditional antibiotics in the fight against AMR.

This study involves screening multiple sites from five canines in a bid to identify novel antimicrobial peptides which target a range of clinically relevant bacteria with a focus on drug-resistant pathogens particularly from veterinary settings.

[P30] GUT MICROBIOTA COMPOSITION OF CHILDREN WITH DIARRHOEA IN JIMMA , ETHIOPIA

Getnet Tesfaw[1], Dawd S. Siraj[2], Alemseged Abdissa[3], Zeleke Mekonnen[3], Mike Zangenberg[4], Jørgen Kurtzhals[5], Kurt Hanevik[6], Oystein Johansen[6], Nina Langeland[7], Nasia Safdar[2], Abigail C. Mapes[2], Ashley Kates[2], Dennis Sandris Nielsen[8]

[1] *University of Copenhagen, Copenhagen, Denmark*
[2] *University of Wisconsin, Department of Medicine, Division of Infectious Diseases, Madison, United States*
[3] *Jimma University, School Medical Laboratory Sciences*
[4] *University of Copenhagen, Department of Immunology and Microbiology*
[5] *Centre for Medical Parasitology, Copenhagen, Denmark*
[6] *University of Bergen, Department of Clinical Science, Addis Ababa, Ethiopia*
[7] *University of Bergen, Department of Medicine, Bergen, Norway*
[8] *University of Copenhagen, 1958 Frederiksberg C, Denmark*

Introduction: Diarrhoea claimed 1.6 million lives in 2016 and about 90% of deaths in under-five children occurred in sub-Saharan Africa and Asia. Childhood mortality associated with acute diarrhoea (AD) is decreasing, but childhood mortality associated with persistent diarrhoea (PD) remains high in LMICs. Persistent diarrhoea accounts for about 10% all diarrhoeal episodes but is responsible for 36% to 54% of all diarrhoea-related mortality. Recent development in metagenomics has showed the myriad role of the gut microbiota on diarrhoea.

Objectives: This study aims to characterize the gut microbiota of children with diarrhoea and to compare disease states to healthy controls.

Methods: A total of 1469 children 0-59 months old of which 799 with diarrhoea and 670 healthy children were recruited at Jimma University Specialized Hospital and Serbo Health Center in Ethiopia and from communities surrounding health facilities, respectively. Fecal samples and clinical data were collected. DNA was extracted from fecal samples at the University of Bergen using standard procedures and shipped on dry ice to the University of Wisconsin for sequencing. The bacterial component of the gut microbiota will be assessed using 16S rRNA target amplicon sequencing by using the Illumina MiSeq sequencing platform bioinformatics and data analysis carried out in well-established bioinformatics pipelines at University of Copenhagen. Analysis based on zero-radius OTUs (zOTUs). In addition, alpha diversity measures such as Shannon and Simpson indices and richness through ACE (abundance-based coverage estimator) and Chao indices will be generated. Subsequently, Bray-Curtis dissimilarity between samples will be determined and clinical data correlated with the microbiota using Redundancy Analysis (RDA).

Expected Results: The study on the gut microbiome analysis will document the gut microbiome compositional differences among children with diarrhoea compared to health children. Furthermore, determine clinical variables that contribute the gut microbiome dysbiosis in Ethiopian children with diarrhoea. Results of the study will give insight for designing clinical managements of diarrhoea based on probiotic and prebiotics.

[P31] SUPPLEMENTING SOW FEED WITH A FERMENTED RAPESEED AND SEAWEED (EP199) PRODUCT MODULATES SOW GUT MICROBIOTA BEYOND FARM SPECIFIC CONDITIONS

Nilay Budeyri Gokgoz[1], Lukasz Krych[1], Yan Hui[2], Rikke Matthiesen[3], Ninfa Pedersen[3], Dennis Sandris Nielsen[1]

[1] *University of Copenhagen, 1958 Frederiksberg C, Denmark*
[2] *University of Copenhagen, Copenhagen, Denmark*
[3] *Fermentationexperts A/S, Bække, Denmark*

Fermented feed attracts attention as an alternative to antibiotics and zinc oxide-based feed supplements for alleviating microbiota-relevant health problems in sows and their offspring. Maternal microbiome modulation through intervention with fermented feed supplements is one strategy to improve gut health. However, the effect of fermented feed on the gut microbiome of sows under varying farm conditions remains largely unknown. In this study, we assessed the impact of fermented rapeseed and seaweed (EP199) on the gut microbiome of 871 sows from 30 Danish pig farms. Fecal samples were collected before and 5-6 months after introduction of the feed supplement. The gut microbiome composition was determined by Oxford Nanopore 16S rRNA gene amplicon sequencing. Our findings showed that farm condition was a key factor in shaping the sow gut microbiome, but also that EP199 inclusion in the feed resulted in sow gut microbiome changes. Overall the number of observed species as well as the Shannon diversity index increased after introduction of EP199. Changes in the relative abundance of specific taxa were also observed, but in a farm-dependent manner. In conclusion, this study shows that farm specific factors strongly influence the sow gut microbiome, but also that by including an adequate number of farms it is indeed possible to identify intervention specific effects consistently. Further, the strategy employed in the present study opens new avenues to manage microbiota-relevant diseases in pigs by applying farm-specific dietary interventions.

SESSION: ENRICHMENT OF PLANTBASED FOODS VIA FERMENTATION

[P32] COMPETITIVE EXCLUSION IS A MAJOR BIOPROTECTIVE MECHANISM OF LACTOBACILI AGAINST FUNGAL SPOILAGE IN FERMENTED MILK PRODUCTS

Solvej Siedler[1], Martin Holm Raua[1], Susanne Bidstrupa[1], Justin M. Ventob[2], Stina Dissing Aunsbjerg Nielsen[3], Elleke F. Bosmaa[4], Laura F. McNaird[4], Chase L. Beiselb[2,5,6], Ana Rute Nevesa[1]

[1] Discovery, R&D, Chr. Hansen A/S
[2] Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695, USA
[3] Global Application, Chr. Hansen A/S
[4] Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, Denmark
[5] Helmholtz Institute for RNA-based Infection Research (HIRI), Helmholtz Center for Infection Research, Würzburg, Germany
[6] Medical Faculty, University of Würzburg, Würzburg, Germany

A prominent feature of lactic acid bacteria (LAB) is their ability to inhibit growth of spoilage organisms in food, but hitherto research efforts to establish the mechanisms underlying bioactivity focused on the production of antimicrobial compounds by LAB. We show in this study, that competitive exclusion, i.e, competition for a limited resource by different organisms, is a major mechanism of fungal growth inhibition by lactobacilli in fermented dairy products. The depletion of the essential trace element manganese by two Lactobacillus species was uncovered as the main mechanism for growth inhibition of dairy spoilage yeast and molds. A manganese transporter (MntH1), representing one of the highest expressed gene products in both Lactobacilli, facilitates the exhaustive manganese scavenging. Expression of the mntH1 gene was found to be strain-dependent, affected by species coculturing and growth phase. Further, deletion of the mntH1 gene in one of the strains resulted in loss of bioactivity, proving this gene to be important for manganese depletion. The presence of a mntH gene displayed a distinct phylogenetic pattern within the Lactobacillus genus. Moreover, assaying the bioprotective ability in fermented milk of selected Lactobacilli from ten major phylogenetic groups identified a correlation between the presence of mntH and bioprotective activity. Thus, manganese scavenging emerges as a common trait within the Lactobacillus genus, but differences in expression result in some strains showing more bioprotective effect than others. In summary, competitive exclusion through ion depletion is herein reported a novel mechanism in LAB to delay growth of spoilage contaminants in dairy products.

[P33] XYLITOL FERMENTATION BY YEAST FROM OIL PALM EMPTY FRUIT BUNCH VIA ENZYMATIC HYDROLYSIS USING CRUDE XYLANASE

Efri Mardawati[1], Roostita Balia[2], Dwi Wahyudha Wira[2], Edy Suryadi[3]

[1] Universitas Padjadjaran, Jatinangor, Indonesia
[2] Dept of Veterinary Medicine, Universitas Padjadjaran
[3] Dept of Agro-Industrial Technology, Universitas Padjadjaran

Oil Palm Empty Fruit Bunch (OPEFB) mainly composes of cellulose, hemicelluloses, lignin and extractive materials. Xylose is the main fraction in the hemicelluloses and it can be converted into xylitol, a natural sugar that is highly valued by the food, chemical and pharmaceutical industries. Xylitol has the sweetness level similar to sucrose but has lower calorie. It is an important sugar substitute for diabetics patients. It has a substantially low viscosity and negative heat of solution causing the cold sensation and it is safe for teeth. The reduction of xylose to xylitol can be performed chemically via the hydrogenation of xylose at high pressures and temperatures by reacting pure xylose with hydrogen gas using a metal catalyst. This process requires pure xylose as the raw material. Alternatively, the reduction process can be carried out via fermentation. This study proposed the production of xylitol from OPEFB via a combination of enzymatic hydrolysis and fermentation process. The availability of xylanase is still limited and its price in the commercial market is very expensive. This research started from the evaluation of xylanase production via solid-state fermentation using OPEFB as substrate and use the crude enzyme to hydrolize hemicellulose in OPEFB to be xylose. Xylose hydrolyzate was used as the substrate for xylitol fermentation. The study and optimization of xylitol fermentation were performed to obtain the maximum yield of xylitol. The optimum fermentation condition then was applied to the xylitol fermentation giving highest xylitol concentration. The fermentation process then was modelled using the Monod kinetic for growth and Luedeking Piret model for xylitol formation.

[P34] IN SITU BETA-GLUCAN PRODUCTION OF LACTIC ACID BACTERIA IN SOURDOUGHS

Julia Bockwoldt[1], Leonie Stahl[1], Johanna Fellermeier[1], Matthias A. Ehrmann[1]

[1] *Technische Universität München, Freising, Germany*

Homopolysaccharide (HoPS) production by lactic acid bacteria (LAB) during sourdough fermentations has widely been studied for an improvement of bread texture and reduction of staling. However, most, HoPS are produced from sucrose by extracellular glucan- or fructansucrases, and residual sucrose causes sweetness of the products. Different from sucrose-derived HoPS-forming LAB, use of β-glucan-forming LAB offers the possibility for bread structure improvement without residual sweetness as β-glucan is formed from naturally available carbohydrates, e.g. maltose in the sourdough, via UDP-glucose. As structure formation is based on capsule-cell networks rather than secreted polysaccharides, low β-glucan amounts promise large structural effects. In this study, we demonstrated the ability of β-glucan forming *Levilactobacillus brevis* TMW 1.2112 and *Pediococcus claussenii* TMW 2.340 isolated from breweries and β-glucan-deficient mutants *L. brevis* TMW 1.2320 and *P. claussenii* TMW 2.2123 to assert in wheat and rye sourdoughs upon two back slopping steps. Further, analysis of various fermentation parameters influencing the β-glucan yield in sourdoughs demonstrated that changes in the fermentation temperature were related to higher β-glucan yields. Rheological experiments revealed that wheat sourdoughs fermented with β-glucan producing *L. brevis* TMW 1.2112 were significantly more viscous compared with wheat doughs fermented by *P. claussenii* TMW 2.340 and the applied mutant strains. In conclusion, such strains can be exploited in a novel generation of bacterial β-glucan-containing sourdoughs for breads with improved structure and water binding, retarded staling, and possibly even health-related added value functions related to those described for plant derived β-glucans.

Part of this work was funded by the project AiF 20462 BG.

[P35] LACTIC ACID BACTERIA FERMENTATION AS A TOOL TO IMPROVE THE ANTIOXIDANT PROPERTIES OF BREWERS' SPENT GRAIN: BIOPROCESS SET-UP, CHARACTERIZATION AND APPLICATION IN PASTA MAKING

Michela Verni[1], Rosa Schettino[1], Annika Krona[2], Rossana Coda[3], Carlo Giuseppe Rizzello[1]

[1] *Department of Soil, Plant and Food Science, University of Bari Aldo Moro, Bari, Italy*
[2] *Rise, Research Institutes of Sweden*
[3] *University of Helsinki, Helsinki, Finland*

Brewers' spent grain (BSG), the main by-product of the brewing industry, contains high concentration of fibers and polyphenolic compounds, which are mostly bounded to cell wall material. Despite its functional potential, BSG is used as low value feed or discarded as waste. Aiming at promoting its use in functional food industry, thirty-two LAB were screened for the ability of improving BSG antioxidant activity during fermentation. *Lactobacillus plantarum* PU1 and PRO17 and *Lactobacillus brevis* H46 caused the most relevant increases and were selected for further experiments. A biotechnological protocol for BSG treatment was set-up and optimized through the addition of a commercial xylanase. Bioprocessed BSG showed high inhibition of linoleic acid oxidation, comparable to that of BHT, and protective effect on human keratinocyte cell lines, comparable to that of **α-tocop**herol. The phenolic profile of raw and bioprocessed BSG was investigated through RP-HPLC-ESI-QTOF. Bound phenolic profile was characterized mostly by phenolic acids and their oligomers. Bound phenols in raw BSG resulted almost 50-fold higher than free phenols. The treatment with xylanase caused the release of 25% of the total bound phenols in free forms. An intense metabolic activity on phenolic acids was observed in fermented BSG leading to the increase of dihydrocaffeic and phloretic acids. Potentially bioactive peptides, showing the typical chemical features of antioxidant sequences, were purified and identified in BSG fermented with *Lb. plantarum* PU1. Peptides encrypted in native cereal proteins were released thanks to the proteolytic activity of the lactic acid bacteria used as starter for fermentation.

Bioprocessed BSG (bBSG), treated with xylanase and fermented with *Lb. plantarum* PU1, was used for pasta making, at 15% substitution level of semolina. bBSG pasta had high content of fibers (ca. 8%), with more soluble fibers compared to raw BSG pasta. An integrated approach including the determination of the main technological, nutritional, and sensory properties was used for the pasta samples characterization.

[P36] ENRICHMENT OF TARHANA IN CONJUGATED LINOLEIC ACID: A NOVEL TURKISH FERMENTED PLANT-BASED FUNCTIONAL FOOD

Koka Zongo[1], Fehmi YAZICI[2], Ahmet Hilmi ÇON[2]

[1] *Ondokuz Mayis University, Samsun, Turkey*
[2] *Department of Food Engineering, Ondokuz Mayis University, Tr-55139 Samsun – Tur-Key*

Nowadays, it is established that malnutrition and foodborne diseases are causative factors of humans' death, economic losses and poverty's increase in the world. During the last century, great progress has been made to improve food safety and the nutritional status of food to strengthen human beings' life. Consequently, over the last two decades, there is increasing stakeholders' interest in new research areas, like functional foods which the implementation of promising results will mitigate both disasters. Thus, taking into account the contrast between the health benefits of conjugated linoleic acid (CLA) and its low content of some foods, added to people's inaccessibility to foods with high content, we aim to enrich with this nutrient *Tarhana*, one of the plant-based staple foods in Turkey.

To achieve this goal, 538 colonies were isolated from the rumen of goat, cheeses, sausages, and fermented *Chloroscombrus chrysurus (Adjuevan)* originated from India, Turkey, and Ivory Coast to test their abilities to produce CLA. Hereby, 30 isolates producing CLA were characterized that 5 belonging to *Lactobacillus spp* and *Enterococcus spp* were used as starter cultures to produce CLA enriched *Tarhana*. The results confirmed that the enrichment of staple foods, other than milk products, with CLA, remains a new approach to impart to consumers the nutritional and functional properties ascribed to this essential nutrient.

[P37] DEVELOPMENT OF STARTER CULTURES FOR FERMENTATION OF PLANT-BASED PROTEIN INGREDIENTS

Nadja Larsen[1], Pernille Johansen[1], Lene Jespersen[1]

[1] University of Copenhagen, Frederiksberg, Denmark

Despite the fact that fermented foods are recognized for their sustainable production, durability, and health-promoting benefits, microbial fermentation is only to a limited extent applied to plant-based protein ingredients. The main objective of this study is to develop microbial starters for fermentation of plant-based protein ingredients in order to improve texture-creating properties, taste, masking of off-flavors and nutritional value. Experiments were performed with food isolates and type strains of bacteria and yeasts (10 strains in total), including *Corynebacterium glutamicum*, *Hafnia alvei*, *Bacillus subtilis* Natto, *Bacillus amyloliquefaciens, Lactiplantibacillus paraplantarum*, *Limosilactobacillus fermentum*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, and *Kluyveromyces marxianus*. The microorganisms were investigated for their ability to grow on protein ingredients from pea, faba beans and soybeans (four protein ingredients in total with a protein content of 55-70%). For growth experiments, the strains were inoculated in water suspensions (7-10% w/v) at initial levels of 4.8-5.8 log CFU/ml for bacteria and 4.6-4.8 log CFU/ml for yeasts and incubated at optimal conditions for 48-72 hours. All microorganisms were able to grow on the protein ingredients reaching stationary phase mostly after 24 h incubation. Viable counts at the end of incubation were in a range of 7.4 – 9.1 log CFU/ml for bacteria and 5.8 – 8.2 log CFU/ml for yeasts, depending on the product and the strain. The highest CFU/ml was obtained for *L. paraplantarum* (9.0 log CFU/ml), *L. fermentum* (8.8-9.1 log CFU/ml), *H. alvei* (8.9 log CFU/ml) and *S. cerevisiae* (7.8-8.2 log CFU/ml) propagated in pea and faba bean based protein ingredients. Controlled fermentations are currently performed to reveal the effect of potential starter cultures on production of volatile compounds, umami-related compounds and sensory characteristics. The ongoing activities are accomplished within the project FERMPRO (Sustainable Production of Fermented Protein Ingredients), which aims to provide consumers with high-quality plant-based protein ingredients by use of innovative pretreatments and processing.

SESSION: OTHERS

[P38] OPTIMIZED SENSORY PROPERTIES OF STEVIA INFUSIONS BY FERMENTATIVE MODIFICATION WITH SINGLE AND CO-CULTURES OF YEAST AND BACTERIA

Claudia Borgmeier[1], Alice Cancellieri[2], Zafer Gezgin[2], Paul Jones[2], Roberto Vanin[2], Juergen Eck[3], Caroline Saunders[2], Guido Meurer[1]

[1] Brain Biotech AG, Zwingenberg, Germany
[2] Lucozade Ribena Suntory Ltd.
[3] Bio.Impact

The leaves of Stevia rebaudiana are known for their sweet taste, which stems from the presence of steviol glycosides that are between 30 and 350 times sweeter than sucrose [1]. However, the use of stevia as a sweetener in its natural form is very limited due to a range of undesirable sensory properties: bitter, metallic taste, astringent mouthfeel and a lingering liquorice aftertaste. They are nevertheless an attractive sugar substitute, because they are heat-stable and do not induce a glycemic response. Approaches to overcome the sensory limitations include the extraction and enzymatic modification of steviol glycosides [2]. In this way, glycosides with low abundance in plant material but offering a favourable taste profile are obtained with the help of recombinant enzymes. However, use of enzymes originating from genetically modified organisms also has drawbacks since consumers prefer to avoid such products [3].

In the present study, we explored an alternative approach by applying single and co-cultures of bacteria and yeasts for the fermentation of crude stevia infusion. One of our targets was to address biochemical changes occurring through microbial activity. The other was to explore the capability of the fermentation process to transform an infusion with strong off-tastes into an ingredient delivering a clean sweetness profile in a target beverage background. To this end, we assessed fermentative metabolites and steviol glycoside by HPLC and evaluated the sensory profiles of samples based on appropriate dilutions in water and fruit juice based drinks. A surprising finding of the biochemical assessment was the absence of substantial changes in relative proportions of steviol glycosides with the exception of specific increases identified for minor derivatives such as Rubusoside. Sensory evaluations on the other hand revealed substantial amelioration of the overall flavour profile in target beverages including low scores in artificialness and lingering as well as delivering cleaner sweetness and mouthfeel.

[1] Brandle, J.E.; Telmer, P.G. Phytochemistry. 2007 Jul;68(14):1855-63.
[2] Gerwig, G.J.; Te Poele, E.M.; Dijkhuizen, L.; Kamerling, J.P. Adv Carbohydr Chem Biochem. 2016;73:1-72.
[3] Ariana Saraiva, A.; Carrascosa, C.; Raheem, D.; Ramos, F.; Raposo, A. Int J Environ Res Public Health. 2020 Aug 28;17(17):6285.

[P39] RISK ASSESSMENT - IDENTIFICATION OF COLIFORM BACTERIA IN DRINKING WATER AT WATER-LINK ANTWERP BY USE OF MALDI-TOF-MS

Hilde Van Winckel[1], Bonner Ann[1], Short Ann[1], Nuyts Hilde[1], Pieter Joos[1]

[1] Water-Link - Laboratory, Mechelsesteenweg 111, B-2840-Rumst and University of Antwerp, Department of Bioengineering Science, Groenenborgerlaan 171, B-2020-Antwerp

Introduction: Water-link produces drinking water for the city of Antwerp and surrounding communities. The laboratory of water-link controls the quality of this drinking water accordingly to the drinking water directive[1]. The objective is to protect human health from adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean. The microbiological parameter coliform bacteria is used as an indicator for possible faecal contamination. Further identification of the coliform bacteria found in the different samples of drinking water gives valuable information for risk assessment.

Materials and Methods: The analysis of coliform bacteria in drinking water is performed on a daily basis. Samples are taken at the exit of the production plant and at the tap. For risk assessment samples are taken along different points in the production process and after working activities on distribution pipes. The samples are analysed for coliform bacteria by one of the following methods. The first method, according to ISO 9308-2, is a most probable number method (Colilert®-18 (IDEXX, Ludwigsburg, Germany)). The second method, according to ISO 9308-1, is based on membrane filtration and incubation (Chromocult® coliform agar (Merck, Darmstadt, Germany)). Since 2017 identification of samples containing coliform bacteria is performed using Maldi-TOF-MS type Microflex LT (Bruker Daltonics, Billerica MA, USA). The Direct Transfer Method as described by the manufacturer was used in combination with the Maldi Biotyper® Compass and Filamentous Fungi Library.

Discussion: Since the absence of coliform bacteria is requested for a good quality of the drinking water only minor amount of samples were found positive (<5%). An inventory of all identified species, total or per SESSION, will be presented. For risk assessment the species were divided over their origin (aquatic or non-aquatic). The share with aquatic origin was high for samples at the tap (62%) and lower for samples after working activities on distribution pipes (27%).

References: [1]Drinking water directive - Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.
ISO 9308 Water Quality – Enumeration of Escherichia coli and Coliform Bacteria, Geneva: International Standards Organization.

[P40] EFFICIENT PRODUCTION OF A-ACETOLACTATE BY WHOLE CELL CATALYTIC TRANSFORMATION OF FERMENTATION-DERIVED PYRUVATE

Robin Dorau[1], Lin Chen[2], Jianming Liu[2], Peter Ruhdal Jensen[2], Christian Solem[2]

[1] Technical University of Denmark, Kgs. Lyngby, Denmark
[2] National Food Institute, Technical University of Denmark, Denmark

Production of food ingredients from biological resources using fermentation or biocatalysis is attractive, as such approaches benefit both the consumer and the environment by avoiding toxic chemicals during the process. Nowadays, the application of highly optimized microorganisms allows the bio-based production of a plethora of chemicals in high amounts and good yields usually using sugars as starting materials. As an example, diacetyl has been produced by fermentation and naturally provides the buttery aroma in products such as butter and margarine. In dairy products it is normally formed spontaneously from αacetolactate, a compound produced in small amounts by selected lactic acid bacteria in the starter culture used. Alternatively, αacetolactate can also be converted efficiently into diacetyl by metal ion catalysis. Due to its bacteriostatic properties, it is difficult to produce diacetyl at high level by fermentation. By expressing a robust αacetolactate synthase (ALS) in a metabolically optimized *Lactococcus lactis* strain we constructed a wholecell biocatalyst that is able to efficiently convert pyruvate into αacetolactate. Under optimized conditions, a titer for αacetolactate of 172 ± 2 mM was achieved using this bioprocess. We then developed a sugar-based twostage αacetolactate production process using an engineered *L. lactis* strain to produce pyruvate from sugar, and subsequently applying the wholecell biocatalyst to convert pyruvate into αacetolactate. Using this approach, we obtained 122 ± 5 mM and 113 ± 3 mM αacetolactate from glucose or lactose in dairy waste, respectively. The wholecell biocatalyst appeared robust and was fully active in crude fermentation broth containing pyruvate. Due to the anaerobic conditions used for the biotransformation, little diacetyl was generated, and this enabled the efficient biotransformation of pyruvate into αacetolactate with the highest titers reported to date. Summarizing, we present an efficient approach for producing αacetolactate from sugar, via pyruvate. We suggest that this two-stage strategy is suitable for converting lactose in dairy waste into αacetolactate and subsequently into diacetyl at larger scale.

[P41] AN S-LAYER GENE-BASED PCR-DGGE ASSAY FOR PROFILING LACTOBACILLUS HELVETICUS STRAIN-LEVEL POPULATION IN WHEY STARTER CULTURES OF GRANA PADANO CHEESE

Francesco Miragoli[1], Vania Patrone[1], Francesco Romaniello[1], Annalisa Rebecchi[1], Maria Luisa Callegari[1]

[1] Università Cattolica del Sacro Cuore, Cremona, Italy

Natural starter cultures are typically used in the manufacture of Italian Protected Designation of Origin (PDO) cheeses such as Grana Padano with the main purpose of fermenting lactose and producing acidification of milk. Among the dominant bacterial components, *L.helveticus* plays a key role both in milk acidification and cheese maturation. In the dairy plant environment, the abundance of individual strains can vary significantly due to daily whey propagation and/or to changes in production parameters influencing the technological capacity of natural starters. Thus, at the industrial level, it is important to monitor *L.helveticus* strain population in whey cultures, e.g. during the propagation process. In this study, we aimed at developing a PCR-denaturing gradient gel electrophoresis (PCR-DGGE) assay based on the analysis of the *slpH* locus. For this purpose, we designed new sets of primers targeting the *slpH* locus, in order to amplify a suitable gene region for PCR-DGGE analysis which could enable discrimination between strains. Since *L.helveticus* strains can be divided in 3 different groups based on the *slpH* locus sequence, we designed three primer pairs, one for each specific group. Our PCR-DGGE method proved to be a rapid and reliable approach for the profiling of *L. helveticus* strain-level population in whey starter cultures. All bands detected in the PCR-DGGE profiles from whey samples showed nucleotide sequences with high similarity to the sequences of *L. helveticus* S-layer genes. Moreover, they perfectly matched with the *slpH* locus sequences of dominant strains obtained by culturing. In conclusion, these results indicated that our PCR-DGGE analysis can provide an accurate picture of *L.helveticus* strain population in whey starters, the target region of the *slpH* locus being sufficiently heterologous for strain discrimination. Moreover, the PCR-DGGE method was able to detect also strains that were missed by conventional culture-dependent techniques, thereby allowing a more comprehensive assessment of whey starter biodiversity.

[P42] WHAT MAKES A "CULT-WINE"? PRODUCTION-CHAIN MICROBIAL DIVERGENCES OF VINEYARDS AND WINES OVER CONSECUTIVE VINTAGES

Alex Gobbi[1], Lars H Hansen[2]

[1] University of Copenhagen, Copenhagen, Denmark
[2] University of Copenhagen, Frederiksberg C, Denmark

"Cult-wines" are expensive, iconic and should represent at the very best the characteristics of the place where they come from within the frame of the "*wine-terroir"*. In our case, the vineyards are located in La Horra, Ribera del Duero, Spain. A place known for the warm and dry climate in summer, where the vines of Tempranillo have been planted and grown for decades, following the traditional "*goblet*" canopy system, in vineyards subjected to biodynamic management. In this scenario, the same winery produces two different wines through spontaneous fermentation, one of which considered being a masterpiece of oenological production. Given the importance attributable to microbes, in all different stages of wine-production, we will focus on their role in differentiating the finished products. Therefore, we will highlight the microbial differences occurring along the wine production-chain, from vineyards to finished wine, over three consecutive vintages, combining amplicon-based NGS and metabolomics profiling of the fermentation samples. We expect to see whether the microbial communities, living in two nearby vineyards, are different at harvest time, or if the communities, initially similar, diverge during the process, generating a measurable effect during the fermentation. The samples collected at the end of the alcoholic and malolactic fermentation will be further sequenced to bin or close genomes of expected unique strains of *Saccharomyces cerevisiae* and *Oenococcus oeni*. The whole genome sequencing will be performed through a hybrid approach combining Illumina high quality short-reads and Nanopore long-read sequencing technologies. So far, during the vintage 2016, only minor differences in relative abundance were detected between the soil samples collected in the two vineyards. However, during the vinification in 2016, major differences arises at the early stage of the fermentation process. These differences, attributable to a non-Saccharomyces low-fermenting yeast such as *Lachancea thermotolerans*, could explain some of the organoleptic features that distinguished the two wines in that specific year. Finally, the outcome of this work may shed some light on the time-stability of the elusive "wine-*terroir"* while identifying new strains of oenological interest for the wine industry.

[P43] CATEGORIZING HISTAMINE PRODUCING BACTERIA FROM GOUDA CHEESE ACCORDING TO AMINO COMPOUND PROFILES

Cleide Oliveira de Almeida Møller[1], Fergal Patrick Rattray[2], Elif F. Ücok[2]

[1] University of Copenhagen, Frederiksberg, Denmark
[2] University of Copenhagen, Copenhagen, Denmark

Alternatives for time consuming and labour demanding microbiological analysis are always required in order to optimize the identification of isolates. This approach is suggested as an alternative for classification of isolates according to the amino compound profiles obtained when investigating the ability of cheese isolates to produce histamine. For this purpose, seven histamine-forming isolates obtained from commercially available long-time-ripened Gouda cheese were selected. The rates of consumption of histidine and formation of histamine were monitored applying different scenarios to investigate the effect of: 1) the presence of glucose (in MRS broth) and absence of glucose (in restricted media with a pH indicator but without glucose added); 2) concentrations of histidine (0.1 and 1.0 %); and 3) incubation time (45, and 120h). Despite the fact that all isolates were obtained from the same vintage Danish Gouda cheese, made from raw cow milk, the amino-compound profile as well as the response to different environmental conditions diverged between the isolates. Rep-PCR and 16S rDNA gene sequencing were used to further characterize the isolates. When comparing the histamine forming behaviour of the tested isolates in restricted media without glucose, it was found that the presence of glucose in the MRS broth completely inhibited histamine production for the *Lentilactobacillus parabuchneri* KUH5, KUH6 and KUH7 isolates. Interestingly, the proportion of histamine formed in relation to the proportion of histidine consumed remained about the same independently of the level of histidine tested. Histamine production was faster for isolates *L. parabuchneri* KUH2, KUH8 and KUH3, compared to *Lb. parabuchneri* KUH1. Based on these results, three categories are proposed: 1) fast producers, *Lb. parabuchneri* KUH3 and KUH8. 2) medium producers, Lb. *parabuchneri* KUH1, KUH2 and KUH6; and 3) slow producers, *Lb. parabuchneri* KUH5 and KUH7. The presence of the histidine decarboxylase gene (*hdcA*) was confirmed by PCR amplification of the histidine decarboxylase gene in four of the isolates. The results indicate that evaluating the presence and concentration of histamine is not only a relevant parameter to evaluate quality and safety, but is also an important tool to classify histamine producers in cheese.

[P44] EVALUATION OF THE EFFECT OF BIFIDOBACTERIUM LACTIS BL-99 ON VOLATILE AND NON-VOLATILE COMPOUNDS OF YOGURT WITH LACTOBACILLUS PARACASEI

Wen Zhao[1], Hsien Liu[1], Jinfeng Liu[1], Wei-Lian Hung[1], Qihe Chen[2], Hongyun Lu[2]

[1] Innovation Center, Inner Mongolia Yili Industrial Group Co.,Ltd, Shanghai, China
[2] Department of Food Science and Nutrition, Zhejiang University, Hangzhou

The objective of this study was to unveil insights into the effects of added *Bifidobacterium lactis* BL-99 on the development of volatile compounds and metabolites in yogurt which was fermented by 2 *Lactobacillus paracasei* strains. Changes in volatile and non-volatile compounds in yogurt fermentation by different combinations of *L. paracasei* strains (K56 and ET-22) were comparatively studied using SPME-GC-MS. It was found that addition of *B. lactis* BL-99 showed a favorable effect on 16 volatile compounds and octanal, butyric acid and caproic acid were significantly increased. Interestingly, BL-99 was found to decrease the formation of lactate in yogurt fermentation by *L. paracasei* strains. In the non-volatile compounds, β-D-galactopyranose formation was increased in the fermented milk added with BL-99. Based on the metabolite analysis, this study characterized the fermentation features of a yogurt added with a *B. lactis* strain BL-99, which may be useful for the production of a tailored yogurt.

[P45] SURVEY OF LACTIC ACID BACTERIA FOR DETOXIFICATION OF PATULIN

Kiymet Guven[1]

1 Eskisehir Technical University, Eskisehir, Turkey

Patulin is often found in many fruits and their products as a toxin. It poses a serious threat to human health due to eating the foods contaminated by patulin. So it is very important to remove or detoxify patulin in foods. There are physical, chemical and biological methods with advantages and disadvantages for removing or degrading it. It has been known that bacteria have the potential for patulin detoxification. Previous studies proved the use of *Gluconobacter oxydans, Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium* for patulin adsorbtion. Therefore, this study was carried out to isolate lactic acid bacteria from different pickles for patulin detoxification.

24 home made and commercial pickle samples varying in their vegetable and salt content (3-8 %) were collected. Lactic acid bacteria isolations were carried out on MRS agar plates and 73 different isolates were collected, purified, identified and then stored as in pure cultures for future use. Both traditional cultural, biochemical and modern techniques were used for identication of the cultures. VITEC, FAME and ribotyping results showed that mainly *Streptococcus* spp., *Lactobacillus* spp*., Alicyclobacillus* spp. and *Leuconostoc* spp. are dominant bacteria in the pickles.

The effects of viable and nonviable (inactivated by autoclave) cells of these LAB isolates will be prepared for biological detoxification of patulin. The detoxification capacities of the strains will be investigated for the first time for the biological detoxification of patulin.

Key words: Patulin, Detoxification, Lactic Acid Bacteria

[P46] MICROBIOTA AND METABOLIC PROFILE OF KEFIR MILK PRODUCED WITH GRAINS AND LYOPHILIZED COMMERCIAL STARTER CULTURES

Maria Barbara Pisano[1], Flaminia Cesare Marincola[2], Maria Elisabetta Fadda[1], Maura Deplano[1], Federica Mereu[1], Gabriella Loghi[1], Sofia Cosentino[1]

[1] University of Cagliari, Dept. Medical Sciences and Public Health, Monserrato (Ca), Italy
[2] University of Cagliari, Dept. Chemical and Geological Sciences, Monserrato (Ca), Italy

Kefir is a fermented milk beverage traditionally obtained from kefir grains, while it is produced commercially by using pure freeze-dried commercial starter cultures with a defined composition and, generally, a low biodiversity compared to grains.

Recently, kefir consumption has increased worldwide, due to its nutritional and therapeutic properties. Kefir grains and milk are characterized by a diverse and complex microbiota, in which lactic acid bacteria, acetic acid bacteria, and yeasts have been reported as predominant. This mixture of microorganisms coexists and interacts to produce a drink fermented product from milk with enhanced nutritional and functional properties due to the transformation of substrates and formation of bioactive compounds responsible for aroma quality and functional properties of this product.

The aim of this study was to compare the microbiota of kefir milk produced with grains with that of milk fermented with lyophilized commercial starter cultures and investigate the biochemical changes due to microbial activity during fermentation. Conventional culturing and a culture-independent approach using high-throughput sequencing of the V3-V4 region of the 16S r RNA gene (Illumina MiSeq) were used to analyze kefir microbiota, while the pool of hydrophilic low molecular weight metabolites (mainly composed by amino acids, organic acids, and carbohydrates) was characterized by means of ^{1}H NMR spectroscopy.

Our results confirmed the complex microbiological composition of kefir and highlighted some differences both from a quantitative and qualitative point of view between the two types of kefir analyzed. The most abundant bacterial genus in Kefir obtained with lyophilized commercial starter cultures was *Lactococcus* (96.5%), followed by *Acetobacter* (2.1%) and *Pseudomonas* (1.1%). *Lactococcus* (56.2%) and *Acetobacter* (36.8%) predominated in Kefir produced with grains, followed by *Lactobacillus*, *Pseudomonas* and *Leuconostoc*. Comparing the NMR metabolic profile of fermented milk evidenced higher levels of acetoin, acetic acid and succinic acid in samples prepared with commercial starters and higher contents of lactose, citric acid, choline, glycerophosphocholine and glutamate in fermented samples obtained with kefir grains. No differences were observed in the content of lactic acid.

[P47] QUANTITATIVE PHYSIOLOGY AND PROTEOME ADAPTATIONS OF BIFIDOBACTERIUM BREVE NRBB57 AT NEAR-ZERO GROWTH RATES

Angela Ortiz Camargo[1], Oscar van Mastrigt[1], Roger Bongers[2], Ben Amor Kaouther[2], Tjakko Abee[3], Eddy J. Smid[3]

[1] Wageningen University & Research, Wageningen, Netherlands
[2] Danone Nutricia Research, Utrecht, Netherlands
[3] Wageningen University, Wageningen, Netherlands

In natural environments, nutrients are usually scarce causing microorganisms to grow slow while staying metabolically active. This is the case in the human gut which harbors a dense population of microorganism generating a low concentration of nutrients despite its constant inflow. These natural conditions where microorganism grow at very low growth rates can be simulated using retentostat cultivations. The present study describes the physiological and proteome adaptations of the probiotic *Bifidobacterium breve* NRBB57 from high (0.4 h^{-1}) to near-zero growth rates. Lactose-limited retentostat cultivations were carried out for 21 days in which the bacterial growth rate was progressively reduced to 0.00092 h^{-1}, with a 3.4 fold reduction of maintenance energy requirement. Lactose was mainly converted into acetate, formate and ethanol at high growth rates while in the retentostat also lactate was produced. Morphological changes and viable but non-culturable cells were also observed in the retentostat. Proteomes were compared for all growth rates, revealing a down-regulation of ribosomal proteins at near-zero growth rate and an up-regulation of proteins involved in the catabolism of alternative energy sources matching the shift in metabolism and providing a basis for the suggested catabolic pathways. Finally, we observed induction of the stringent response and stress defence systems, including chaperons involved in protein and DNA repair. Retentostat cultivations were proven useful to study the physiology of *B. breve*, mimicking the nutrient scarcity of its natural habitat, the human gut.

[P48] ANALYSIS OF STRAIN DIVERSITY WITHIN COMMERCIAL BIOPROTECTIVE LACTICASEIBACILLUS RHAMNOSUS CULTURES

Elif F. Ücok[1], Finn Vogensen[1], Fergal Patrick Rattray[1]

[1] University of Copenhagen, Copenhagen, Denmark

Yeast and mold are important causes of spoilage in dairy products, and cheese is an excellent substrate for these organisms to grow in. Spoilage due to yeast and mold can cause quality and food safety problems that may result in economic loss. Furthermore, several cheese-contaminating mold species produce mycotoxins that can be hazardous to humans. The best method for control of these unwanted organisms in dairy products is preventing contamination and inhibiting their growth. A promising solution for inhibiting their growth is the use of lactic acid bacteria (LAB). Various antimycotic compounds including many organic acids, cyclic dipeptides, and fatty acids can be produced by LAB. *Lacticaseibacillus rhamnosus,* which is a homofermentative, non-spore-forming lactic acid bacterium may be used as a protective culture against yeast and mold growth. In this work, we studied the genetic diversity of different commercial cultures of *L. rhamnosus*. Six different commercial *L. rhamnosus* protective cultures from four different suppliers were compared using Repetitive (REP) sequence-based polymerase chain reaction (PCR) using the $(GTG)_5$ 5′-GTGGTGGTGGTGG-3′primer. The DNA fingerprints were analyzed by Bionumerics software using the Dice Similarity coefficient. We observed that the strain numbers of *L. rhamnosus* cultures differed from one to three. Based on the strain types and clusters that were defined by DNA banding pattern similarities, a single strain F culture showed 95% similarity with E culture which contained two strains of *L. rhamnosus*. In addition, a single strain B culture showed 98% similarity with C culture which consisted of three strains of *L. rhamnosus*. However, a single strain D culture showed 61% similarity with A culture which consisted of two strains of *L. rhamnosus*. Our study revealed that *L. rhamnosus* supplied by different companies exhibit diversity to a moderate extent at the genetic level.

[P49] IN VITRO PLATFORM TO STUDY MICROBIOME METABOLISM OF POLYPHENOLS

Maria Wiese[1], Brandon Iker[2], Matt Runyon[2], Frank Schuren[1]

[1] Tno, Netherlands
[2] Amway, USA

Introduction: The bioavailability of PPs is overall low, and the gastrointestinal tract plays a vital role in the absorption, distribution, metabolism, and excretion of PPs. Some microbes utilize and convert PPs in the gut (Aravind et al., 2021, Loo et al., 2020). However, the lack of direct access to the intestine restricts studying the bioconversion of PPs *in vivo*.

Materials & Methods: We have combined our in vitro intestine screening platform, the i-screen, with a state-of-the-art advanced biochemical analytical platform (LC-MS) to unravel the interactions of PPs with the gut microbiota. In this study, we have investigated the potential bioconversion of quercetin, cyanidin-3-glucoside, epigallocatechin, hesperidin, ellagic acid, daidzein, and secoisolariciresinol diglucoside by incubating these with 11 individual intestinal microbiome samples from healthy donors.

Results: Metabolism of 6 out of 7 polyphenols was detected within 24h. Metabolism was clearly microbiome dependent, with different microbiome donors showing varying metabolic potential. Both disappearances of parent compounds and the appearance of metabolites could be detected.

Conclusions: Our study demonstrates the possibility of gaining insights on microbial bioconversion of PPs *in vitro*. Furthermore, our experimental platforms and read-outs can guide the optimization of PP bioavailability and function.

References

Aravind, S. M., Wichienchot, S., Tsao, R., Ramakrishnan, S. & Chakkaravarthi, S. 2021. Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. Food Research International, 110189.

Loo, Y. T., Howell, K., Chan, M., Zhang, P. & NG, K. 2020. Modulation of the human gut microbiota by phenolics and phenolic fiber-rich foods. Comprehensive Reviews in Food Science and Food Safety, 19, 1268-1298.

[P50] HUMAN-FIRST PROBIOTICS DISCOVERY USING ULTRA-HIGH-RESOLUTION MICROBIOMICS

Henrik Bjørn Nielsen[1]

[1] *Clinical-Microbiomics, København N, Denmark*

Classical, probiotics discovery starts by studying the properties of a microbe *in vitro*. Typically, an isolated *Bifidobacterium* or *Lactobacillus* strain is screened for production and colonisation potential as well as hazard risk, through a series of *in vitro*, *in silico* and *in animal* tests. Ultimately, the microbe is tested in humans for safety, and only then for efficacy. Instead, we propose a human-first approach, linking desired human phenotypes, like absence of disease or fast recovery, to clades of naturally occurring microbial populations. In this way insight from discovery cohorts may point to possible application areas, even before strains have been isolated and tested. Moreover, integration of existing strains allows novel applications for existing probiotic to be found. This is made possible by ultra-high resolution microbiomics and phylogenetic analysis across large data collections.

Notes

Poster overview

No.	Title	Presenter
P01	The effect of direct-fed microbials on grass or maize silage on in-invitro rumen fermentation	Rajan Dhakal
P02	High-throughput phenotyping of 239 Leuconostoc strains	Vera Kuzina Poulsen
P04	Bioactivity and sensory perspective of probiotic yogurt fortified with apple pomace flour	Marina Jovanović
P05	Pre-pro-postbiotics and role in modulating our microbiome - status & case studies	Radhika Bongoni
P06	The effect of pectins on adhesion of Limosilactobacillus fermentum PCC and integrity of the Caco-2 cell monolayer	Thanyaporn Srimahaeak
P07	Crafting fermentation nutrients is key for developing efficient & industry-ready culture media for probiotics & microbiome-based strains manufacturing	Micheline El Khoury
P08	In vitro studies of the effects of Saccharomyces cerevisiae on epithelial barrier integrity compromised by Candida albicans 3135	Thanyaporn Srimahaeak
P09	Tasting the terroir of wine yeast innovation	Sakkie Pretorius
P10	Influence of nitrogen source on expression of genes involved in aroma production in Saccharomyces uvarum	Angela Coral Medina
P11	Strain diversity in vitamin B1 yield in Saccharomyces cerevisiae	Rebecca Rocchi
P12	Optimal conditions for virome sample preservation revealed by bacteriophage infectivity and virome metagenomic analysis	Xichuan Zhai
P13	Extracellular production of polysaccharide-degrading-enzymes by Mucor sp. through fermentation of brewers spent grain	Shady Hassan
P14	A multi-enzyme carbohydrase product improves fiber solubilization of cereals and oil seeds	Eduardo Antonio Della Pia
P15	Muramidase induced peptidoglycan depolymerization in vivo is measured by novel LCMS-MS method quantifying the concentration of soluble- and total muramic acid in digesta	Mads Pedersen
P16	Cell surface attachment of the Lactococcus lactis PrtP protease	Egon Bech Hansen
P17	Water kefir represents a reservoir for lactic acid bacteria encoding novel glucansucrases	Julia Bechtner
P18	Malt flavour in Swiss semi-hard cheese	Ueli von Ah
P19	How to screen for agents that prevent late blowing defect in cheese	Sofia Dashko
P20	Lactococcal extracellular membrane vesicles deliver bioactive vitamin K2 to human cells	Yue Liu
P21	Effect of a dual-purpose silage inoculant on pathogenic Escherichia coli O157:H7 in grass silage	Audrey Segura
P22	Studying the effects of faecal microbiota spent medium on intestinal mucin production - method development	Grete Raba
P23	Isolation of commensal bacteria from piglet small intestine	Sami Aideh
P24	Selection of probiotic strains for microbiome priming in pigs	Lea Hübertz Birch Hansen
P25	Developing methods for microbiome analysis for animal health studies	Radhika Bongoni
P26	Characterization of the vaginal DNA virome in health and dysbiosis: An opening study in patients with non-female factor infertility	Rasmus Riemer Jakobsen
P27	In vitro immune modulatory effect of a thymol and cinnamaldehyde blend and organic acids	Chong Shen
P28	Vitamin B12 and bacterial microcompartments stimulate the anaerobic growth of Listeria monocytogenes on rhamnose	Zhe Zeng
P29	Man's best friend: antimicrobial compounds isolated from bacterial strains of canine source.	Michelle O' Connor
P30	Gut Microbiota Composition of Children with Diarrhoea in Jimma , Ethiopia	Getnet Tesfaw
P31	Supplementing sow feed with a fermented rapeseed and seaweed (EP199) product modulates sow gut microbiota beyond farm specific conditions	Nilay Budeyri Gokgoz
P32	Competitive exclusion is a major bioprotective mechanism of lactobacili against fungal spoilage in fermented milk products	Solvej Siedler
P33	Xylitol Fermentation By Yeast from Oil Palm Empty Fruit Bunch Via Enzymatic Hydrolysis using Crude Xylanase	Efri Mardawati
P34	In situ beta-glucan production of lactic acid bacteria in sourdoughs	Julia Bockwoldt

No.	Title	Presenter
P35	Lactic acid bacteria fermentation as a tool to improve the antioxidant properties of brewers' spent grain: bioprocess set-up, characterization and application in pasta making	Michela Verni
P36	Enrichment of Tarhana in Conjugated Linoleic Acid: A novel Turkish fermented plant-based functional food	Koka Zongo
P37	Development of starter cultures for fermentation of plant-based protein ingredients	Nadja Larsen
P38	Optimized sensory properties of stevia infusions by fermentative modification with single and co-cultures of yeast and bacteria	Claudia Borgmeier
P39	Risk assessment - identification of coliform bacteria in drinking water at water-link Antwerp by use of MALDI-TOF-MS	Hilde Van Winckel
P40	Efficient production of a-acetolactate by whole cell catalytic transformation of fermentation-derived pyruvate	Robin Dorau
P41	An S-layer gene-based PCR-DGGE assay for profiling Lactobacillus helveticus strain-level population in whey starter cultures of Grana Padano cheese	Vania Patrone
P42	What makes a "Cult-Wine"? Production-chain microbial divergences of vineyards and wines over consecutive vintages	Alex Gobbi
P43	Categorizing histamine producing bacteria from Gouda cheese according to amino compound profiles	Cleide Oliveira de Almeida Møller
P44	Evaluation of the effect of Bifidobacterium lactis BL-99 on volatile and non-volatile compounds of yogurt with Lactobacillus paracasei	Wen Zhao
P45	SURVEY OF LACTIC ACID BACTERIA FOR DETOXIFICATION OF PATULIN	Kiymet Guven
P46	Microbiota and metabolic profile of kefir milk produced with grains and lyophilized commercial starter cultures	Maria Barbara Pisano
P47	Quantitative physiology and proteome adaptations of Bifidobacterium breve NRBB57 at near-zero growth rates	Angela Ortiz Camargo
P48	Analysis of strain diversity within commercial bioprotective Lacticaseibacillus rhamnosus cultures	Elif F. Ücok
P49	In vitro platform to study Microbiome metabolism of polyphenols	Maria Wiese
P50	Human-first probiotics discovery using ultra-high-resolution microbiomics	Henrik Bjørn Nielsen

Author index

BOLD = PRESENTING AUTHOR

Name	No.
Abdissa, Alemseged	P30
Abee, Tjakko	P47, P20
Acin Albiac, Marta	**O33**
Adamberg, Kaarel	P22, **O01**
Adamberg, Signe	P22, O01
Aideh, Sami	**P23**
Alexandra, Baumeyer Brahier	P18
Ann, Bonner	P39
Ann, Short	P39
Appels, Rudi	O08
Arike, Liisa	P22
Arneborg, Nils	O08
Aslampaloglou, Antonios	P12
Bachmann, Herwig	O14
Balia, Roostita	P33
Béal, Catherine	P03
Bechtner, Julia	**P17**
Bernfried Biron, Mathias	O25
Bianchi, Fernanda	P06
Bockwoldt, Julia	**P34**
Boin, Gilles	O19
Bongers, Roger	P47
Bongoni, Radhika	O12, P25
Bongoni, Radhika	**P05**
Borgmeier, Claudia	**P38**
Borne, Johanna	O31
Borodina, Irina	**O25**
Bourdichon, Francois	**O18**
Brockmann, Elke	P02
Bruggeman, Frank	O14
Budeyri Gokgoz, Nilay	**P31**
Butler, Derek	O12
Butler, Derek	P05
Cagno, Raffaella di	O33
Callegari, Maria Luisa	O30, P41
Calvo, Estefania Pérez	P15
Camarasa, Carole	P10
Cancellieri, Alice	P38
Caprarulo, Valentina	O30
Castex, Mathieu	O13
Castro Mejia, Josue L.	P26, P12
Cerelus, Bram	O15
Cesare Marincola, Flaminia	P46
Chen, Lin	P40
Chen, Qihe	P44
Christensen, Ib	O26
Christensen, Lise Friis	**O23**
Cieplak, Tomasz	P23
Coda, Rossana	O33, O34, P35
ÇON, Ahmet Hilmi	P36
Copani, Giuseppe	P23, P21, P01
Coral Medina, Angela	**P10**
Cosentino, Sofia	P46
Costessi, Adalberto	**O12**
Cotter, Paul D.	O03
Darbani, Behrooz	O25
Dashko, Sofia	**P19**
Daures, Celia	O31
Del Cortone, Andrea	O15
Della Pia, Eduardo Antonio	**P14**
Dell'Anno, Matteo	**O30**
Demuyser, Liesbeth	P08
Deng, Ling	P26, P12
Deplano, Maura	P46
Dhakal, Rajan	**P01**
Dorau, Robin	**P40**
Duijsings, Danny	O12
Eck, Juergen	P38
Ehrmann, Matthias A.	P34
El Khoury, Micheline	**P07**
Engels, Wim	198
Fadda, Maria Elisabetta	P46
Fellermeier, Johanna	P34
Fidiarakis, Philippos	P14
Field, Des	P29
Filannino, Pasquale	O33
Fonseca, Fernanda	P03
Frederiksen, Carsten Østergaard	P15
Gallo, Antonio	P21
Gallone, Brigida	O15
Garcia Moreno, Pedro Jesus	O26
Geronikou, Athina	**O09**
Gezgin, Zafer	P38
Gobbetti, Marco	O33
Gobbi, Alex	**P42**
Gorjanović, Stanislava	P04
Gram, Lone	O13
Gregersen, Simon	O23, **O26**
Greisen, Gorm	O05
Gu, Fangjie	**O04**
Guinane, Caitriona	O03
Guven, Kiymet	**P45**
Haahr, Thor	P26
Hanevik, Kurt	P30
Hansen, Egon Bech	O23, O26, **P16, O22**
Hansen, Hanne Helene	P01
Hansen, Lars H	P42
Hansen, Lea Hübertz Birch	**P24**
Hansson, Gunnar C.	P22
Hassan, Shady	**P13**
Hejna, Monika	O30
Hélène, Meng Yi	P18
Herody, Caroline	O19
Herrera-Dominguez, Lucia	P02
Hilde, Nuyts	P39
Hill, Colin	O27, P29
Holck, Jesper	O31
Holm Rau, Martin	**O17**
Howell, Kate	O08
Hui, Yan	O31, **O05**, P31
Humaidan, Peter	P26
Hung, Wei-Lian	P44
Iker, Brandon	P49
Jacobsen, Charlotte	O26
Jakob, Frank	P17
Jakobsen, Rasmus Riemer	**P26**
Janssen, Renske	198
Jariana, Abbas	O15
Jensen, Jorgen Skov	P26
Jensen, Kristian	**O11**
Jespersen, Lene	P08, O09, P06, P37, O04, **O06**
Johansen, Oystein	P30
Johansen, Pernille	P37
Jones, Paul	P38
Joos, Pieter	P39
Jorgensen, Katrine	P27
Jovanović, Marina	**P04**
Kadekar, Darshana	P27
Kaouther, Ben Amor	P47
Kates, Ashley	P30
Katina, Kati	O34
Kell, Douglas	O25
Khakimov, Bekzod	O31
Kjærulff, Søren	O36
Klaassen, Paul	P19
Klausen, Mikkel	P15
Koirala, Prabin	**O34**
Kot, Witold	P26
Kragh, Karsten	P27
Krishna Prabhala, Bala	O25
Krogfelt, Karen Angeliki	O05
Krona, Annika	P35
Krych, Lukasz	O05, P23, P31
Kurtzhals, Jørgen	P30
Laforce, Ida Nynne	P16
Laitila, Arja	O02
Laličić Petronijević, Jovanka	P04
Langeland, Nina	P30
Larsen, Nadja	P08, O09, P06, **P37**, O04
Leser, Thomas Dyrmann	P26
Lillevang, Søren Kristian	O09
Liu, Hsien	P44
Liu, Jianming	P40
Liu, Jinfeng	P44
Liu, Sijin	P19
Liu, Yue	**P20**
Loghi, Gabriella	P46

Author index

BOLD = PRESENTING AUTHOR

Name	No.
Lopez-Ulibarri, Rual	P15
Lu, Hongyun	P44
Luis, Ana Sofia de Jesus Vaz	P22
Maas, Evy	O31
Maina, Ndegwa Henry	O34
Mapes, Abigail C.	P30
Marcatili, Paolo	O26
Mardawati, Efri	**P33**
Marr, Beinta	O04
Matthiesen, Rikke	P31
Maukonen, Johanna	O02
May, Ali	**P25**
Medina, Jacqueline	O25
Mekonnen, Zeleke	P30
Mereu, Federica	P46
Meurer, Guido	P38
Michelsen, Birgit	O04
Milora, Nina	P21, P01
Miočinović, Jelena	P04
Miragoli, Francesco	O30, P41
Mireille, Tena Stern	P18
Mitić-Ćulafić, Dragana	P04
Molenaar, Douwe	O14
Morell, Indrek	P22
Morrissey, John	P10
Mortensen, Martin Steen	O05
Møller, Frank	**O10**
Nielsen, Dennis Sandris	O31, O05, P12, P30, P31
Nielsen, Dennis Sandris	P26
Nielsen, Henrik Bjørn	**P50**
Nihtilä, Hanna	O34
Ntokou, Eleni	O26
O' Connor, Michelle	**P29**
O`Sullivan, Orla	O03
Oeregaard, Gunnar	P02
Oliveira de Almeida Møller, Cleide	**P43**
Olsen, Tobias Hegelund	O26
Ortiz Camargo, Angela	**P47**
Ouwehand, Arthur	**O02**
Overgaard, Michael Toft	O23, O26
Pascal, Fuchsmann	P18
Pascale, Nélida	O04
Patrick Rattray, Fergal	P43, P48
Patrone, Vania	**P41**
Pedersen, Helene	P27
Pedersen, Mads	**P15**
Pedersen, Ninfa	**O36**, P31
Pénicaud, Caroline	P03
Perez-Samper, Gemma	O15
Perret, Bruno	P03
Petersen, Sune Allan	O04
Petrović, Marija	P04
Piampongsant, Supinya	O15
Pisano, Maria Barbara	**P46**
Planqué, Bob	O14
Poulsen, Vera Kuzina	**P02**
Prandini, Aldo	O30
Pretorius, Sakkie	**P09**
Raba, Grete	**P22,** O01
Randelovic, Milica	O25
Rebecchi, Annalisa	P41
Reggi, Serena	O30
Remo S., Schmidt	P18
Respondek, Frederique	O04
Rizzello, Carlo Giuseppe	O33, P35
Rocchi, Rebecca	**O07**
Romaniello, Francesco	P41
Ross, Paul	P29
Rossi, Luciana	O30
Ruhdal Jensen, Peter	P40
Runyon, Matt	P49
Safdar, Nasia	P30
Salminen, Seppo	**O24**
Saunders, Caroline	P38
Schettino, Rosa	P35
Schneider, Hannah	P22
Schuren, Frank	P49
Schwab, Clarissa	**O29**
Sébastien, Dubois	P18
Segura, Audrey	**P21**
Sejberg, Jimmy J.P.	O31
Shen, Chong	**P27**
Siedler, Solvej	**P32**
Siraj, Dawd S.	P30
Smid, Eddy J.	P47, P20, O07
Smith, Birgitte	O05
Solem, Christian	P40
Sonnenschein, Eva	**O13**
Steensels, Jan	O15
Sørensen, Jens Fribæk	**O20**
Sørensen, Søren Johannes	O05
Sotira, Stefania	O30
Sourabié, Alain M.	**O16**, P07
Spinnler, Henry Eric	P03
Srimahaeak, Thanyaporn	**P08**, **P06**
Stahl, Leonie	P34
Streekstra, Hugo	P19
Suryadi, Edy	P33
Tesfaw, Getnet	**P30**
Teusink, Bas	**O14**
Thymann, Thomas	O31
Tovborg, Morten Broch	P14
Ücok, Elif F.	P43, **P48**
Van Den Bogert, Tom	O12
van der Hoek, Steven	O25
van der Meulen, Bernd	**O19**
van Dijk, Albert	**O21**
Van Immerseel, Filip	**O28**
van Mastrigt, Oscar	P47
van Ophem, Eline	P20
Van Winckel, Hilde	**P39**
Vanin, Roberto	P38
Vermeersch, Lieselotte	O15
Verni, Michela	**P35**
Verstrepen, Kevin	**O15**
Vogel, Rudi F.	P17
Vogensen, Finn	P48
von Ah, Ueli	**P18**
Voordeckers, Karin	O15
Wahyudha Wira, Dwi	P33
Warda, Alicja	**O27**
Wells-Bennik, Marjon	198
Wiese, Maria	**O31**, **P49**
Winters, Michela	**O08**
Wosinska, Laura	**O03**
Wösten, Han	**O32**
YAZICI, Fehmi	P36
Yesiltas, Betül	O26
Zangenberg, Mike	P30
Zeng, Zhe	**P28**
Zhai, Xichuan	**P12**
Zhao, Wen	**P44**
Zlatanović, Snežana	P04
Zongo, Koka	**P36**
Zugaj, Karolina	O25

Notes

Thank you to our partners, sponsors & supporters

PARTNERS

CHR. HANSEN — Improving food & health

DSM — BRIGHT SCIENCE. BRIGHTER LIVING.

LALLEMAND — LALLEMAND BIO-INGREDIENTS

novozymes — Rethink Tomorrow

GOLD SPONSORS

iff — Where science & creativity meet

SILVER SPONSORS

BASECLEAR

BECKMAN COULTER — Life Sciences

KPM ANALYTICS

Samplix

BRONZE SPONSORS

Aage Christensen as

sbi SCIENTIFIC BIOPROCESSING

BioSense Solutions

CONSTANT SYSTEMS Ltd — Cell Disruption Made Easy

GPKelco — A HUBER COMPANY

unibio

Yakult

TECAN.

SUPPORTERS

CARLSBERG FOUNDATION

Federation of European Microbiological Societies

MIFFI