Book of Abstracts

Expert Workshop on

”Microbial Diversity & Health”

IFF, Leiden, Netherlands

7 – 8 June 2023

LABIP Expert Workshop ”Microbial Diversity & Health”

Program

Wednesday June 7th
Arrival at IFF between 12.00-13.00. A small sandwich lunch will be provided

13.30: Start of the Workshop

- **LABIP intro**
  - Objective of the workshop: The impact of microbial diversity in food on health — **Esben Laulund**
- **Can beneficial microbes play a role in promoting diversity related to the hygiene hypothesis?**
  - Microbial diversity and its relevance to food and health — **Paul Cotter**
  - What are the consequences of food processing evolution on food microbes diversity? — **Gaspar Perez**
  - Next-Gen Fermented Food: Harnessing Gut Microbiome Diversity and Functions — **Julien Tap**

Q&A panel discussion

15.30-16.00 Tea, coffee

- **How to influence microbial diversity in today’s Western diet?**
  - Biotics as microbiota modifiers: definitions and future directions — **Seppo Salminen**
  - Effects of fermented food consumption on human gut microbiome diversity using Kefir as an example — **Liam Walsh**
  - Elucidating effects of fermented dairy food on intestinal gene expression, microbiome composition and metabolic activity — **Louise Jakobsen**

Q&A / panel discussion

18.00 Drinks and dinner at the Hilton hotel

Thursday June 8th

9.00: Start 2nd day Workshop

- **The consumer perspective**
  - Probiotic microorganisms and European consumers’ perception: key findings of the survey “Do you know what probiotics are?” — **Rosanna Pecere**
- **Can food microbes play a key role in the diversity of the human microbiome?**
  - Bacteriophages – A key component in shaping the gut microbiome — **Torben Rasmussen**
  - Selective inactivation of food microbes- emerging process solutions and sustainability performance — **Alexander Mathys**
  - Old friends: the potential of fermented foods and probiotics — **Olaf Larsen**

11.00-11.30 Tea, coffee

Q&A / panel discussion

WEBSITE & COMMUNICATION

13.00 Closure of the Workshop. A small sandwich lunch will be provided

Participants LABIP Expert Workshop “Microbial Diversity & Health”

Speakers

Paul Cotter	Teagasc Food Research Centre
Louise Jakobsen	Aarhus University
Olaf Larsen	Yakult
Alex Mathys	ETH Zurich
Rosanna Percere	IPA Europe
Gaspar Perez	C.S.I.C.
Torben Rasmussen	University of Copenhagen
Seppo Salminen	University of Turku
Julien Tap	University Paris, Saclay
Liam Walsh	Teagasc Food Research Centre

LABIP Representatives

Patrice Antoine	THT
Sylvie Binda	Lallemand
Lone Brond-Miller	IFF
Patrick Derkx	Chr. Hansen
Stéphane Duboux	Nestlé R&D
Guus Kortman	NIZO
Jolanda Lambert	NIZO
Esben Laulund	Chr. Hansen
Aat Ledeboer	LABIP
Sophie Legrain	Gnosis by Lesaffre
Daniella Lucena	Arla
Arjen Nauta	FrieslandCampina
Jean-Philippe Obert	IFF
Raish Oozeer	Danone
Tadgh O’Sullivan	Heineken
Jan Willem Sanders	Unilever R&D
Karoline Terberger	Firma E. Böcker KG
Nikoletta Vidra	Yakult

LABIP introduction & Objective of the workshop: The impact of microbial diversity in food on health

Esben Laulund

President LABIP, Vice President, Chr. Hansen, Denmark

The Lactic Acid Bacteria Industrial Platform **LABIP** is an European Economical Association, founded in 1994. The members of LABIP are EU based companies that produce or apply Lactic Acid Bacteria (LAB) and furthermore have production or research facilities within the EU. Currently LABIP has 19 members.

LABIP main activity has been to serve as the Industry Platform for several EU funded Framework program research projects involving LAB.

LABIP has since LAB 8 (2005) been the main sponsor of awards for scientifically excellent & industrially relevant research on LAB presented at the International LAB Symposia under the auspices of FEMS.

LABIP has in recent years focused on organizing consensus expert workshops on various scientific aspects related to the industrial application of LABs LABIP has so far organized 7 expert workshops

Additional information can be found at: www.labip.com

Background for the LABIP Expert Workshop on "Microbial Diversity & Health"

It is estimated by FAO/WHO that up to 1/3 of all the food products consumed by humans have undergone a fermentation process.

Although traditionally fermented foods still have an increasing positive image with consumers, industrialized and pasteurized/sterilized convenience foods have during the recent decades gained a greater foothold within the human diet and consequently the average consumer today is less exposed to microbes from the food products they consume.

The Expert Workshop will address different issues of fermenting microbes present in foods such as Lactic Acid Bacteria and their impact on the general health and wellbeing of humans.

The program of this expert workshop will address the following aspects:

- Can beneficial microbes play a role in promoting diversity related to the hygiene hypothesis?
- How to influence microbial diversity in today's Western diet?
- The consumer perspective on fermented food products
- Can food microbes play a key role in the diversity of the human microbiome?

The aim of this LABIP Expert Workshop, is therefore to discuss the opportunities and challenges that are involved for achieving a natural diversity of beneficial microbes in today's food production and their influence on the health and well-being of humans.

The outcome of the Expert Workshop will be published as a LABIP position paper outlining the opportunities and challenges for the EU industry using Lactic Acid Bacteria to promote microbial diversity and health

Microbial diversity and its relevance to food and health

Paul Cotter

Head of Department, Food Biosciences, Senior Principal Research Officer, Teagasc Food Research Centre, Moorepark, Cork, Ireland

A tremendous variety of fermented foods are produced by all societies globally. However, there are still many fermented foods that have yet to undergo in-depth microbiome analysis to reveal the diversity of species and strains present therein. An even smaller subset of fermented foods have been the focus of pre-clinical/clinical studies. Despite this, the studies that have taken place show a huge untapped potential. This potential can be achieved through the creation of communities of fermented food microorganisms designed to capture key health promoting, and other, features of fermented foods in a manner that also ensures that highly consistent products can be generated at scale.

What are the consequences of food processing evolution on food microbes diversity

Gaspar Pérez Martínez

Lactic Acid Bacteria and Probiotics Laboratory, Instituto de Agroquímica y Tecnología de Alimentos

Spanish National Research Council (C.S.I.C.), Valencia, Spain

Food Processing involves all possible methods and treatments that transform raw materials into food or facilitate their consumption by humans or animals. Food Processing has three basic aims: changing food properties –nutritional, palatable-, improving safety and shelf life, and achieving accessibility and convenience. Since diet constituents bring essential nutrients to the intestinal microorganisms and allochthonous microbial input interact or enriches gut populations, it is expected that Food Processing will impact on human gut microbiome, and this review aimed to find evidences of it. It has been recently reported that hunter-gatherer populations have different gut microbiome and higher diversity than urban people. Experimental trails performed after adhesion to Paleolithic diet indicated small but significant changes in the diversity and composition of gut microbiota. Cooking is the most basic Food Processing method and it beneficially affects gut microbiota profiles, possibly by the effect of Maillard compounds. Pasteurization and sterilization assays run on animal models improved digestibility but decreased microbial diversity in the gut. A historical perspective of Food Processing highlights that since the II World War heat treatments expanded globally, suggesting a coincidence in time with problems associated to the Hygiene Hypothesis, but there are not enough evidences to support a dominant role of Food Processing in the Hygiene Hypothesis. Certainly child care procedures developed in the last century and dietary habits (i.e. fructose and meat consumption) have robust evidences of their impact on infant's microbiome and health, and they would be major candidates to support the Hygiene Hypothesis.

Next-Gen Fermented Food: Harnessing Gut Microbiome Diversity and Functions

Julien Tap

University Paris, Saclay, France

The gut microbiome is essential for human health, yet modulation requires more insight into inter-individual variation. Recent large cohort studies showed that environmental and host factors explain less than 20% of the variation in microbial composition, suggesting significant roles for stochastic factors and ecological rules in gut microbiome assembly. Although diet and lifestyle significantly influence the symbiosis between the host and microbiome, resilience is identified as a critical factor in maintaining the microbiome's structure throughout an individual's lifespan.

Understanding the microbiome structure included analyzing 35,000 human gut microbiome samples and using time-series analysis. We found that the microbiome is structured as branches that shape dynamics between states. Gut microbiome states are differentially linked to host and environmental factors, allowing critical transition to unhealthy degraded states to be detected in association with diversity and functional shifts.

As part of a healthy diet, fermented foods hold the potential to counter decreased microbiome diversity and have potential health benefits such as improving gut health. Although there is a significant intersection between food and human microbial species, the origin of fermented foods strains is crucial to consider its functional effect. While consuming fermented foods has been associated with metabolic changes in the gut microbiome, its functional enrichment is considered personalized. This updated view of the human gut microbiome structural and functional landscape provides a conceptual framework for designing next-gen and sustainable fermented foods.

'Biotic' family: definitions and future perspectives

Seppo Salminen, Functional Foods Forum, Faculty of Medicine, University of Turku, Finland

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 sessile 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 nutritional value of fermented foods, leading to the search 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 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)

Given the mechanisms by which microbiota-modulating foods may achieve their desired effects, the current regulatory framework needs definitions that help in understanding the challenges and rewards in determining how microbiota modulating components should be classified. Over the past 10 years, ISAPP (www.ISAPPscience.org) has advanced consensus definitions of probiotics, prebiotics synbiotics and postbiotics. Each definition requires existing evidence of health promoting effects. Consensus definitions are useful for these developing fields, so that scientists, clinical trialists, industry, regulators, and consumers have common ground for future activity in the area of biotics. A generally accepted definition for each member of the biotic family will hopefully help in creating regulatory clarity and promote innovation and the development of new microbiota modulating products. The definitions and their background have been published in Nature Reviews in Gastroenterology and hepatology as open access publications.

ISAPP definitions for fermented food, probiotic, prebiotic, synbiotic and postbiotic:

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)

Effects of fermented food consumption on human gut microbiome diversity using Kefir as an example

Liam Walsh

Teagasc Food Research Centre, Ireland

It has been established that the human gut microbiota is central to health. Disturbance of the gut microbiota has been linked to a variety of diseases, including colon cancer, diabetes, inflammatory bowel disease, and obesity. Consequently, the gut microbiota has emerged as a potential target for the prevention or treatment of such diseases. Targeted manipulation of the gut microbiota can be achieved by dietary intervention, including through the use of fermented foods. Health benefits have also been attributed to several traditional fermented foods and the microbes therein [9]. Particular examples of kefir, a traditional fermented milk product (FMP), have been linked to health benefits, including anti-cholesterolemic, anti-inflammatory, and anti-pathogenic effects [10-12], and several investigations indicate that specific microorganisms in kefir contribute to these effects. Here we discuss recent findings relating to the modulating properties of kefir consumption on the human gut microbiota .

Fermented dairy for bone health: Elucidating effects of fermented dairy food on intestinal gene expression, microbiome composition and metabolic activity

Louise M. A. Jakobsen

Aarhus University, Denmark

Osteporosis is a disease of the bone, where reduced bone mineral density is observed. Osteoporosis is especially prevalent in women aged < 50 years, which due to postmenopause have lower estrogen-levels. Prevalence of osteoporosis is responsible for health care costs of 56.9 billion euros linked to direct costs of incident fractures, long-term disability costs and pharmacological interventions.

Calcium intake is an important determinant of bone health. In Western countries, dairy products are the most important dietary sources of calcium. Calcium absorption occurs through active or passive processes in various segments of the gastrointestinal tract. While the majority (~65 %) is being absorbed in the ileum, around 10 % is absorbed in the colon. Both milk and yoghurt contain a high amount of bioavailable calcium. While the calcium in milk is enclosed in casein micelles, protecting the calcium against low gastric pH in the stomach and enhancing the solubility in the small intestine, yoghurt contain starter cultures that stimulates a healthy gut microbiota, potentially promoting lower-gut absorption of calcium. Inulin is a prebiotic fiber, which, through modulation of the gut microbiota, might also impact calcium absorption. Emerging evidence suggests that intake of fermented dairy such as yoghurt is associated with an increased bone mineral density and a reduced risk of fracture in postmenopausal women. This has led to the proposal of a gut-bone axis, where modification of gut microbiota impacts bone metabolism through a reduction in bacterial-induced inflammatory response on osteoclast-mediated bone resorption and effects on bacterial-derived metabolites involved in the regulation of calcium absorption.

In order to explore the effect of calcium-source, yoghurt and inulin on bone mineralization, we designed a 6-week rat intervention study with a model of postmenopausal women: ovarectomized (OVX) rats. The diets included calcium fortified milk, calcium fortified yoghurt, calcium-fortified yoghurt with inulin and a positive (calcium fortified, no dairy) and negative control (low calcium, no dairy) treatments. After 6 weeks, the rats were sacrificed and examined using whole body DXA, femur bone strength and structure (X-ray and NMR), NMR metabolomics of intestinal content and blood and finally 16S rRNA sequencing of cecum content to determine microbiota composition. Both yogurt and the yogurt-inulin combination had a pronounced effect on the gut microbiome and stimulated *Streptococcus* and *Lactobacillus* on the expense of *Clostridia.* Yogurt as a calcium vehicle was superior to milk to enhance bone mineral density and this was associated with relative abundance of yogurt bacteria. The yoghurt-inulin combination caused gut metabolome modulation (higher short-chain fatty acid concentration) in cecum, colon and feces, which also contributed to a lower pH in intestinal content, however this was not associated with changes in bone mineralization. The beneficial effects of yogurt on bone mineral density may involve glycine-related pathways (glycine, serine and threonine metabolism). Glycine is an important amino acid in type I collagen in bone. To investigate the effects of dairy matrix and prebiotic administration in the gut environment, we designed another 6-week intervention study and examined the metabolome in the gut and plasma as well as transcriptomic of mucus. The results showed that inulin was the most efficient in lowering intestinal pH and that yoghurt appeared to enhance this effect. Furthermore, milk and yoghurt caused markedly different gene expression profiles in gut mucus.

This work is still in process, but so far, we have indications that the gut environment responds very differently to the dairy matrix and to supplementation of prebiotics and this might influence the absorption of calcium to achieve better bone health.

Probiotic microorganisms and European consumers' perception: key findings of the survey 'Do you know what probiotics are?'

Rosanna Pecere

IPA Europe, Belgium

The evolution of European consumers' opinions, trends, and behaviors shows a strong interest in overall health and well-being. It also highlights that consumers would like to be informed about the role of probiotic microorganisms in our daily life. IPA Europe asked to a market research company 3Gem to carry out a survey from a representative sample of 8.000 People in 8 countries of the EU (Italy, Denmark, the Netherlands, Spain, Poland, Belgium, Germany, and Sweden). The key findings give to us some indication of the level the awareness on probiotic foods and food supplements, and on what consumers would like to know more about, when it comes to probiotic food and food supplements.

Bacteriophages – A key component in shaping the gut microbiome

Torben Sølbeck Rasmussen

Section of Microbiology and Fermentation, Dept. of Food Science, University of Copenhagen, Frederiksberg, Denmark

Gut microbiome (GM) composition and function are linked to human health and disease, and routes for manipulating the GM have become an area of intense research. Due to its high treatment efficacy, the use of fecal microbiota transplantation (FMT) is generally accepted as a promising experimental treatment for patients suffering from GM imbalances (dysbiosis), e.g. caused by recurrent Clostridioides difficile infections (rCDI). Mounting evidence suggests that bacteriophages (phages) play a key role in successful FMT treatment by restoring the dysbiotic GM, but widespread use of FMT is unlikely due to inconsistent treatment outcomes and the risk of infection by bacteria and human viruses that are transferred along with the donor material. As a refinement to FMT, fecal virome transplantation (FVT, removing the bacterial component of donor feces by sterile filtration) decreases the risk of invasive infections caused by bacteria. Recent clinical and preclinical studies have demonstrated the potential of FVT as a phage-based therapy targeting different disease etiologies associated to GM dysbiosis, such as rCDI, metabolic syndrome, and necrotizing enterocolitis. I'll discuss the promises of FVT along with the perspectives of developing methodologies to overcome some of its limitations, which may lay the ground for designing novel phage-based therapies for GM restoration.

Selective inactivation of food microbes- emerging process solutions and sustainability performance

Alexander Mathys

ETH Zurich, Institute of Food, Nutrition and Health, Sustainable Food Processing Laboratory, Schmelzbergstrasse 9, Zurich 8092, Switzerland.

Selective inactivation of food microbes can be accomplished by different lethal effects, where selected examples of innovative thermal, electro-magnetic, mechanical, and combined processes will be introduced hereafter.

Ultra-short thermal processes in milliseconds can deliver new inactivation opportunities by using relatively known lethal thermal effects. Such high temperature ultra-short processes benefit from different temperature dependencies of inactivation kinetics in these process windows. Furthermore, modular thermal micro process engineering was effectively applied to improve upscaling of microbial inactivation processes. Electro-magnetic based pulsed electrical field (PEF) processing enables an inactivation of specific microorganisms and species with less thermal effects. One of the reasons, that PEF was successfully implemented as more gentle pasteurization process in the fruit and vegetable juice industry. During mechanical high pressure processing in batch, focused investigations on the property changes within pure water and more complex systems, such as microorganisms, enabled a detailed understanding of the respective process-product-operation interactions. After studying bacterial inactivation in very detail, classical high pressure preservation could be optimized through combined thermal and mechanical processes such as high pressure thermal sterilization as well as continuous ultra-high pressure processing up to 400 MPa as innovative multi hurdle technologies for gentle preservation of healthy and high quality food.

Holistic life cycle sustainability assessment, aligned with the introduced process innovations, can evaluate the suggested solutions on a multi parameter base, in terms of improved food production sustainability.

Old friends: the potential of fermented foods and probiotics

Dr. Olaf F.A. Larsen,

Yakult, Belgium

Adequate gut microbiota management requires a One Health approach. In this presentation, epidemiological data on both communicable and non-communicable diseases will be presented. New data on macronutrient intake will also be presented, demonstrating that these data are not sufficient for an adequate explanation of the rise in metabolic disorders. Altogether, the data will be put in the perspective of a deterioration of the gut microbiota quality which calls for, among others, a targeted intervention using keystone microbial taxa and guilds. Nutrition can be one of the interventional modalities towards such restoration of the gut microbiota quality, but suffers from an inherently low effect size. Therefore, results will be presented focusing on optimizing the statistical power for clinical trials using nutritional intervention. The potential of fermented food stuffs will subsequently be discussed to modulate the gut microbiota. To facilitate a more rational approach towards gut microbiota intervention, a regiment will be proposed that carefully balances between a one-size-fits-all intervention and the supplementation of future personalized microbial consortia. As a first step towards such interventions, computer simulations estimating the ecological dimensions of these future personalized microbial consortia will be presented