



Yakult

International Symposia

Beneficial Microbes

March 27th and 28th, 2025
SÃO PAULO - BRAZIL

Fundamental Science and Innovative Applications

Evento que reunirá especialistas nacionais e internacionais para compartilhar insights das mais recentes descobertas sobre a ciência dos probióticos, abrangendo aspectos de produção e aplicação em alimentos, ração animal, agricultura e medicina.

yakultsymposiumbrazil.com.br

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WYNDHAM®

São Paulo Ibirapuera
Convention Plaza





Program and Book of Abstracts



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Welcome Message from Organizing committee

Helena Sanae Kajikawa – Yakult

Prof. Dr. Svetoslav Dimitrov Todorov – USP

The beneficial properties of microorganisms and their metabolites have been studied for centuries, along with their use as starters or biopreservatives in the manufacture of fermented foods, as well as in the formulation of traditional medicines and cosmetics. All this was based on empirical knowledge passed down from generation to generation over the centuries. However, in early 20th century France, Stamen Grigorov and Élie Metchnikoff suggested that yogurt and its associated lactic acid bacteria could play an important role in promoting health. In Japan, Dr. Shirota was one of the most prominent pioneers in introducing lactic acid bacteria into bioactive health-promoting foods and medicine, based on scientific evidence of the beneficial properties of selected microorganisms.

Now, a century later, we have strong scientific arguments linking the beneficial properties of numerous lactic acid bacteria to various fermented foods. Today, as we enter the next millennium, we are closer to fulfilling Hippocrates' dream that one day our food will become medicine, and our medicine will become food.

Sharing knowledge gained from years of laboratory work and ideas about how this knowledge can be applied to improve human health is the driving force behind organizing this symposium. Yakult's mission "to contribute to the health and happiness of every person around the world through the pursuit of excellence in life sciences and the study of microorganisms" became the

inspiration for this symposium, and the International Probiotic Conferences (IPC) have joined these efforts to make them as interesting and productive as possible. We prove that by working harder we become stronger.



Message from the President of Yakult Brazil - Mr. Atsushi Nemoto



The founder of Yakult, Dr. Minoru Shirota, always believed that the intestine was fundamental to health,

which has been increasingly proven by scientists from different countries. Since its foundation, Yakult has been conducting research with *Lactobacillus casei* Shirota and other beneficial microorganisms, collaborating with various research institutions worldwide and thus obtaining many high-impact scientific discoveries.

The Brazilian branch started its activities in 1968. It was the first unit of the Japanese multinational outside of Asia and is a pioneer in bringing the concept of fermented milk with live lactobacilli to the Brazilian consumer. For this reason, in Brazil, unlike other Western countries, many



people already know and practice the concept of probiotics.

The realization of this symposium in Brazil, in partnership with IPC, reinforces Yakult's commitment to disseminating the benefits of probiotics. Our goal is also to create opportunities for Brazilian researchers to build a network and exchange information with the world's leading researchers on the latest discoveries regarding these

beneficial microorganisms for human health, as well as to provide the development of products that truly bring benefits to the population.

We hope it will be a productive day filled with knowledge.



Message from the President of the IPC - Dr. Norbert Bomba.

“Unlock the Future of Probiotics: Join Us in São Paulo for the Yakult Probiotic Symposium 2025!”

Step into the future of probiotic research and development at the Yakult Probiotic Symposium 2025! Join us in São Paulo, Brazil, from March 27th and 28th for an immersive experience like no other.

Connect with top scientists, medical professionals, and industry leaders driving innovation in gut (and even more) health. Dive into dynamic discussions, interactive workshops, and thought-provoking presentations. Gain valuable insights, forge new collaborations, and stay ahead of the curve in this rapidly evolving field. Our goal is to enhance future collaborations and to work for a better (probiotic) world.

Plus, explore the vibrant culture, culinary delights, and natural beauty of Brazil and São Paulo, adding an unforgettable dimension to your symposium experience.

Don't miss this opportunity to revolutionize your understanding of probiotics and discover the advantages of São Paulo, Brazil!



Norbert Bomba,
International Scientific Conference on

Yakult Beneficial Microbes, 27-28 March 2025, Sao Paulo, Brazil



Probiotics, Prebiotics, Gut Microbiota and Health.

Yakult: A Global Probiotic Pioneer

Yakult is a renowned Japanese company that specializes in the production of probiotic beverages. Founded in 1935 by Dr. Minoru Shirota, Yakult's mission is to contribute to the health and happiness of people worldwide through the pursuit of excellence in life sciences and research on microorganisms. The company's flagship product, Yakult, is a sweetened probiotic milk beverage fermented with the bacteria strain *Lacticaseibacillus paracasei* Shirota. This strain was developed by Dr. Shirota and is known for its ability to survive in the acidic environment of the stomach and reach the intestines alive, where it promotes gut health.

Yakult's commitment to quality and safety has made it a global leader in the probiotic industry. The company offers a range of products, including food and beverages, cosmetics, and pharmaceuticals, all designed to support overall health and well-being. Yakult's products are available in over 40 countries and regions, and the company continues to expand its reach through innovative research and development.



Yakult Brazil: A Local Success Story

Yakult's journey in Brazil began in 1968 when the company opened its first factory outside Japan in São Bernardo do Campo, São Paulo. Since then, Yakult Brazil has become a significant player in the local market, offering a variety of products that cater to the diverse needs of Brazilian consumers. The company's product lineup includes the classic Yakult drink, Yakult 40, Yakult 40 *light*, and a range of other dairy-based beverages and supplements.

Yakult Brazil is dedicated to promoting health and wellness through its high-quality probiotic products. The company actively engages in scientific research and collaborates with local universities and research institutions to advance the understanding of probiotics and their benefits. Yakult Brazil also participates in community outreach programs and educational initiatives to raise awareness about the importance of gut health and the role of probiotics in maintaining overall well-being.

In addition to its commitment to health, Yakult Brazil is also focused on sustainability and environmental responsibility. The company implements eco-friendly practices in its production processes and strives to minimize its environmental impact.



Yakult Beneficial Microbes, 27-28 March 2025, Sao Paulo, Brazil



Organizing committee

Helena Sanae Kajikawa

Yakult S/A Ind. e Com.

Departamento de Ciências e Pesquisas

Prof. Svetoslav Todorov

University of São Paulo, Brazil

Scientific Committee

Prof. Svetoslav Todorov

University of São Paulo, Brazil

Dr. Norbert Bomba

International Scientific Conference on Probiotics, Prebiotics, Gut Microbiota and Health, Slovakia

Prof. Michael Leonidas Chikindas

Rutgers, The State University of New Jersey, USA

Prof. Ana Lúcia Barretto Penna

São Paulo State University, Brazil

Dr. Bruno Pot

Yakult Europe, The Netherlands

Evaluation board

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Prof. Sabrina Casarotti, Brazil

Prof. Santosh Kumar Tiwari, India

Prof. Svetoslav Dimitrov Todorov, Brazil

Prof. Vasco Azevedo, Brazil



General Information

Accommodation

Wyndham São Paulo Ibirapuera Convention Plaza

Av. Ibirapuera, 2927 – Ibirapuera, São Paulo – SP

Promocode: YIS2025

Reservation: <https://reservastrul.com.br/wyndham-sp-ibirapuera/>

Sao Paulo

Discover São Paulo, a vibrant metropolis that never sleeps! Immerse yourself in its rich culture, where world-class museums and theaters meet pulsating nightlife. Savor culinary delights from around the globe in the city's countless restaurants and street markets. Shop till you drop in trendy boutiques and sprawling malls. Marvel at the striking architecture, from historic landmarks to modern skyscrapers. Explore lush parks and green spaces amidst the urban jungle. Whether you're a foodie, art enthusiast, or nightlife seeker, São Paulo offers endless experiences that will captivate your senses. Come and experience the energy and diversity of this dynamic city!

Coffee

Take a break and savor the moment with a cup of premium coffee, *Yakult* and juice at the Yakult Conference in São Paulo this March 2025. As you network with industry experts, there's nothing quite like the aroma and warmth of freshly brewed coffee and premium beverages from the *Yakult* production portfolio to recharge your mind and fuel your creativity. Enjoy rich, smooth flavors while engaging in stimulating conversations. Our coffee break offers the perfect opportunity to relax, refresh, and refocus amidst the bustling energy of the conference. Don't miss out on this delightful experience that blends innovation and relaxation in the heart of São Paulo. Cheers to great coffee and inspiring connections!

Certificates of Attendance

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Certificates will be provided after the conference via the online platform of the event.

Food & Beverages

During the conference, you will have options for meals in the restaurant of the **Wyndham São Paulo Ibirapuera Convention Plaza Hotel** or in the nearby **Ibirapuera Shopping Center**.

Parking

During the conference, you will have park your car at the **Wyndham São Paulo Ibirapuera Convention Plaza Hotel** or in the nearby **Ibirapuera Shopping Center** on your own cost.

Registration desk

Registration desk will be open on 27 and 28 March 2025 from 08h00 till 18h30.

Whether

As March draws to a close in São Paulo, the weather remains comfortably warm, with temperatures typically ranging from 59°F (15°C) to 78°F (26°C). However, it's important to note that this period also brings a significant amount of rainfall. On average, São Paulo experiences around 8 to 15 rainy days in March. The city can receive up to 7.87 inches (200 mm) of rain during this month.

Given the frequent rain showers, it's wise to carry an umbrella to stay dry while exploring the vibrant streets of São Paulo. The rain can be quite unpredictable, with sudden downpours occurring throughout the day. An umbrella will not only keep you dry but also allow you to continue enjoying the city's attractions without interruption.

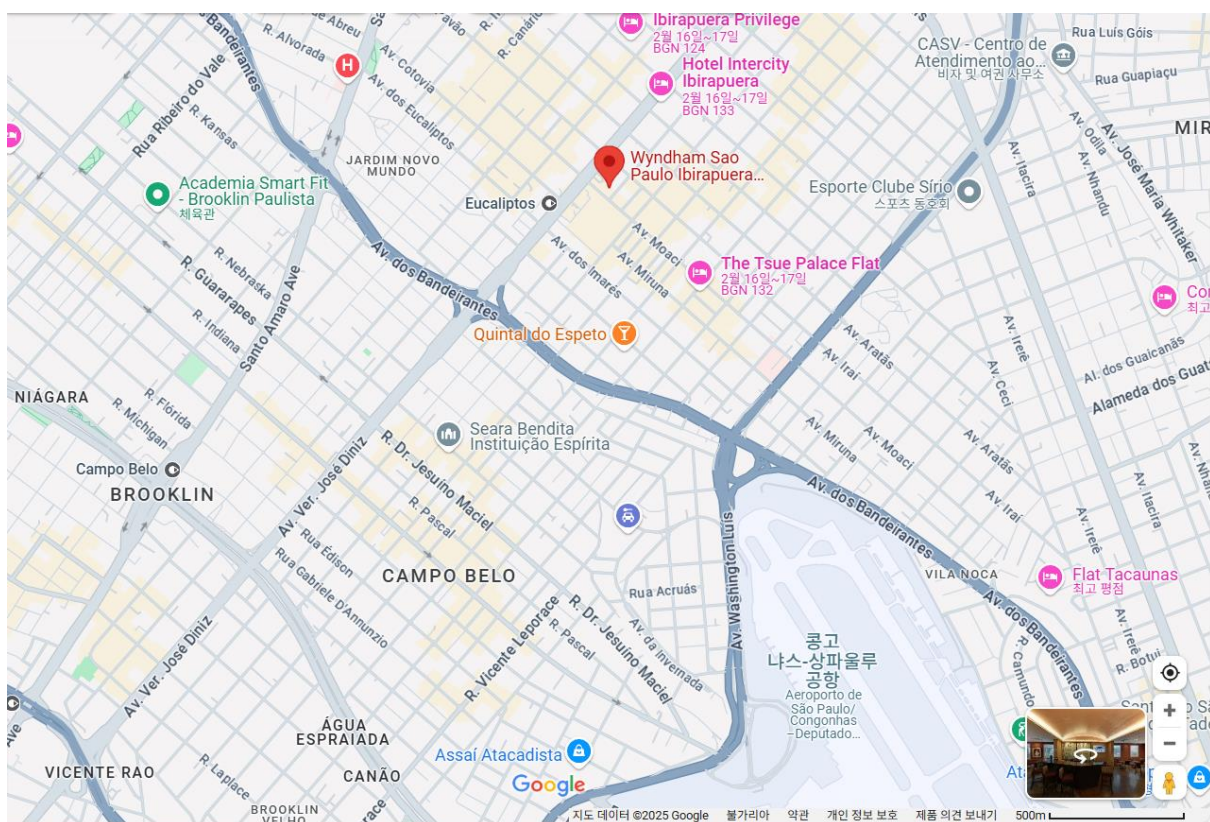
Despite the rain, São Paulo offers plenty of indoor activities to keep you entertained. From world-class museums and art galleries to bustling markets and cozy cafes, there's always something to do. So, whether you're attending a conference, exploring the city's cultural landmarks, or



simply enjoying a stroll, having an umbrella on hand will ensure you make the most of your time in São Paulo, rain or shine.

Venue

Wyndham São Paulo Ibirapuera Convention Plaza Av. Ibirapuera, 2927 – Ibirapuera, São Paulo – SP



Exhibition: "Cerrado, flowers and bees"

The photography exhibition is part of the extension project “The Cerrado and its faces: Raising community awareness about the importance of environmental preservation through art”. This extension action aims to help raise awareness among the population about the importance of preserving the Cerrado and its bees. The awareness process is worked on through poems and

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photographic exhibitions of stingless and stingless bees, in association with native and cultivated flowers of the Cerrado.

Tatiana de Sousa Fiuza, Pedro Vale de Azevedo Brito, Luiza Toubas Chaul, Luiz Carlos Cunha, Virgínia Farias Alves, Pierre Alexandre dos Santos



Scientific program

Day 1 / 27 March 2025

08h00-09h00 **Registration**

09h00-09h30 **Opening Ceremony**

Mr. Atsushi Nemoto, President of Yakult Brazil

Ms. Helena Sanae Kajikawa, Yakult Brazil, co-President of the conference

Prof. Svetoslav D Todorov, Faculty of Pharmaceutical Sciences, University of Sao Paulo, co-President of the conference

Prof. Joilson O. Martins, director of the Faculty of Pharmaceutical Sciences, University of Sao Paulo

09h30-10h15 Moderator: Prof. Svetoslav Todorov

Plenary lecture: Dr. Tatsuichiro Shima, Yakult Central Institute

Characteristics of gut microbiome, organic acid profiles and viral antibody indexes of healthy Japanese with live *Lactocaseibacillus* detected in stool

10h15-11h40 Session: **New horizons for beneficial microbes in human health**

Moderator: Prof. Vasco Azevedo

10h15-10h40 Dr. Hannia Leon, ISLI Mesoamerica, Costa Rica

The Gut Microbiome and Aging. ILSI's Global Scientific Approach

10h40-11h05 Prof. Vasco Azevedo, UFMG, Belo Horizonte, MG, Brazil

Probiogenomics: Genomic analysis of probiotics and their health benefits

11h05-11h30 Prof. Alexander N. Suvorov, Russian Academy of Science, Institute of Experimental Medicine, Saint-Petersburg, Russia

Microbial therapy of infectious and non-infectious diseases, reasons behind and novel approaches for making probiotics

11h30-11h40 Discussion

11h40-13h10 Session: **Beneficial qualities beyond probiotics**

Moderator: Prof. Svetoslav Dimitrov Todorov

11h40-12h00 Prof. Svetoslav D Todorov, FCF/USP, São Paulo, SP, Brazil

Safety evaluations, an essential step in validation of bacteriocin-producing lactic acid bacteria in biotechnological processes

12h00-12h20 Prof. Ana Lúcia Barretto Penna, UNESP, São José do Rio Preto, SP, Brazil

Healthy and tasty food: Innovative applications of probiotic lactic acid bacteria in fermented foods

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- 12h20-12h40 Prof. Santosh Kumar Tiwari, Maharshi Dayanand University, Rohtak, Haryana, India.
Bacteriocin production among lactic acid bacteria: A recently discovered probiotic trait having multifarious applications in human and animal health
- 12h40-13h00 Prof. Ingrid Suryanti Surono, Bina Nusantara University, Jakarta, Indonesia
Functional foods for a healthy microbiome
- 13h00-13h10 Discussion
- 13h10-14h30 Lunch time
- 14h30-15h00 Session: **Future of science: Students talks & selected presentations**
Moderator: Prof. Ana Lucia Barretto Penna
- 14h30-14h40 Gabriele Manamy Baba Rodrigues, ICB - Instituto de Ciências Biomédicas da USP, São Paulo, SP, Brazil
Contextual effect and safety of probiotics consumption in intestinal inflammatory processes
- 14h40-14h50 Kayque Carneiro, FCF/USP, São Paulo, SP, Brazil
Evaluation for optimal conditions of γ -aminobutyric acid production by *Lactiplantibacillus plantarum* ST0414
- 14h50-15h00 Priscila Longo, Universidade São Judas Tadeu, São Paulo, SP, Brazil
Effects of synbiotics, protein supplementation associated to resistance training on inflammation, oxidative stress, and muscle strength in older adults with type 2 diabetes mellitus: A triple-blinded randomized controlled trial
- 15h00-16h30 Coffee & poster session
- 16h30-18h00 Session: Regulation and healthy microbes: match or mismatch?
Moderator: Bruno Pot
- 16h30-17h00 Dr. Bruno Pot, Yakult, The Netherlands
Dietary microbes, a necessary recommendation for healthy eating?
- 17h00-17h25 Prof. Celia Ferreira, Federal University of Vicosa, Vicosa, MG, Brazil
Probiotic and probiotic health claims: Comparative regulatory frameworks
- 17h25-17h50 Dr. Clenio Pillon, EMBRAPA, Brasilia, DF, Brazil
Probiotics for Safe and Healthy foods: Embrapa's "One Health" Program
- 17h50-18h00 Discussion
- 18h30-20h30 Welcome reception



Day 2 / 28 March 2025

- 08h00-09h00 Registration
- 09h00-09h45 Moderator: Prof. Michael Chikindas
Plenary lecture: Dr. Kana Yahagi, Yakult Central Institute
Gut microbiota development and short-chain fatty acid composition in early life
- 09h45-11h20 Session: **Can Beneficial Microbes Boost Your Performance, Mood, and Cosmic Adventure?**
Moderator: Prof. Adriane Elisabete Antunes de Moraes
- 09h45-10h10 Prof. Adriane Elisabete Antunes de Moraes, UNICAMP, Limeira, SP, Brazil
Microbiota modulation and sports performance
- 10h10-10h35 Prof. Viacheslav Konstantinovich Ilyin, Russian Federation State Scientific Center – Institute for Biomedical Problems, Russian Academy of Sciences, Moscow, Russia
Experience of Autoprobiotics Application for Correction Human Microbiota Under Conditions of Isolation and Dry Immersion
- 10h35-11h10 Prof. Andre Bachi, Federal University of São Paulo, Sao Paulo, SP, Brazil
Daily Intake of Fermented Milk Containing *Lactobacillus casei* Shirota (Lcs) – Modulatory effects on Immune and Inflammatory Responses in Marathon Runners
- 11h10-11h20 Discussion
- 11h20-12h00 Session: **New generation in science: Students talks & selected presentations**
Moderator: Prof. Djamel Drider
- 11h20-11h35 Dr. Françoise Coucheney, University of Lille, Lille, France
Local artisanal dairy fermented products as reservoir of beneficial microorganisms
- 11h35-11h50 Dr. Adriano Cruz, Instituto Federal de Rio de Janeiro, Rio de Janeiro, RJ, Brazil
Functional Minas frescal cheese with spore-forming *Weizmannia coagulans* GBI-30
- 11h50-12h00 Marina Moreira de Castro, UNICAMP, Campinas, SP, Brazil
Gut microbiome composition and its association with dietary factors in Crohn's disease



12h00-13h15 Lunch time

- 13h15-15h00 Session: **How they work and what they do**
Moderator: Prof. André Bachi
- 13h15-13h35 Prof. Wilhelm Holzapfel, Handong Global University, Republic of Korea
Discovering functional probiotics protecting against inflammatory Bowel disease
- 13h35-14h00 Prof. Nadiya Boyko, Uzhhorod National University, Ukraine
Microbiome and atherosclerosis biomarkers or how could we prevent age- and stress- relevant diseases individually
- 14h00-14h25 Prof. Gislane Lelis Vilela de Oliveira, UNESP, Botucatu, SP, Brazil
The role of the microbiota-immune system axis in probiotic functions
- 14h25-14h50 Prof. Mauro Walter Veisberg, UNIFESP, São Paulo, SP, Brasil
The role of probiotics and prebiotics in inducing gut immunity
- 14h50-15h00 Discussion

15h00-16h30 Coffee & poster session

- 16h30-17h30 Session: **The power of probiotics: How microbes can enhance animal nutrition and performance**
Moderator: Prof. Michael Leonidas Chikindas
- 16h30-16h50 Prof. Michael L. Chikindas, Rutgers State University, New Brunswick, NJ, USA
Sporeforming probiotics for poultry: what, where, and how?
- 16h50-17h10 Prof. Seyed Hossein Hoseinifar, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
Intensified but sustainable aquaculture, the implication of novel microbial feed supplement
- 17h10-17h30 Prof. Djamel Drider, University of Lille, Lille, France
Role of enterocin DD14 in prophylactic and therapeutic control of necrotic enteritis in broilers using *Enterococcus faecalis* 14 and its Δ bac mutant
- 17h30-17h40 Discussion
- 17h40-18h00 Awards and Closing ceremony
Presentation of the Awards
Mr. Hidenori Yoshitomi, Superintendent Director of Yakult Brazil
Ms. Helena Sanae Kajikawa, Yakult Brazil, co-president of the conference
Prof. Svetoslav D. Todorov, Faculty of Pharmaceutical Sciences, University of Sao Paulo, co-president of the conference



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Awards

“Yakult Award for Young Scientist Best Poster Presentation”, awarded by Yakult do Brasil. Prize in kind and certificate.



IPC – International Scientific Conference on Probiotics, Prebiotics, Gut Microbiota and Health – Slovak Republic, in collaboration with the organizing committee, will present a special award (certificate and voucher for the registration for upcoming IPC2025 Athens 24-26 June 2025) for the best poster presented during the event in the area of Health Promoting Microorganisms.

CISAS – Center for Research and Development in Agrifood Systems and Sustainability, Instituto Politécnico de Viana do Castelo, 4900-347 Viana do Castelo, Portugal, in collaboration with the organizing committee, will present a special award (certificate) for the best poster presented during the event in the area of Applied Food Microbiology.



**Centro de Investigação
e Desenvolvimento em Sistemas
Agroalimentares e Sustentabilidade**
Instituto Politécnico de Viana do Castelo



Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil, in collaboration with the organizing committee, will present a special award (certificate) for the best poster presented during the event in the area of Applied and Fundamental Pharmaceutical Sciences.



Abstracts

Oral presentations

Invited Speakers

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OP01 **Characteristics of gut microbiome, organic acid profiles and viral antibody indexes of healthy Japanese with live *Lacticaseibacillus* detected in stool**

Tatsuichiro Shima

Manager, Senior Researcher, Yakult Central Institute, Yakult Honsha Co., Ltd., Tokyo, Japan

Background

Probiotics are defined as ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’. *Lacticaseibacillus paracasei* (the previous taxonomic nomenclature was *Lactobacillus casei*) YIT 9029 (strain Shirota: LcS) is a popular probiotic strain, and fermented milk products containing LcS (FML) are consumed daily worldwide (www.yakult.co.jp/english/). Several studies have shown the health-promoting effects of LcS intake, including immune regulation, infectious protection, improvement of mental health and sleep quality through the brain-gut axis, and maintenance of gut microbiome composition in the stool. However, it is unclear whether LcS reach the lower gastrointestinal tract alive and contributes to the health-promoting effects described above.

Aim

Our aim was to estimate the health-promoting effects of LcS that reached the lower gastrointestinal tract alive.

Methods

Stool and serum samples were collected from 72 healthy Japanese adults. The characteristics of gut microbiome, organic acid profiles, defecatory symptoms, and serum viral antibody indexes of subjects were analyzed using 16S rRNA amplicon sequence analysis, HPLC, questionnaire survey, and ELISA, respectively. Live LcS was detected using propidium monoazide-quantitative PCR, and these characteristics were compared between the groups in whom live LcS was detected or not from stool.

Results

The β -diversity index of the gut microbiome constituted a significant difference between the live-LcS-detected-group (LLD) and the live-LcS-not-detected-group (LLnD). In the LLD, Bifidobacteriaceae, Lactobacillaceae, and Coriobacteriaceae counts were significantly higher, and the succinate concentration was significantly lower than that in the LLnD. The serum herpes simplex virus (HSV) immunoglobulin (Ig) M antibody index in the LLD tended to be lower than that of the LLnD in HSV IgG-positive subjects. Of the LLD, those in the FML-high-frequency-group (FML-HF) and those in the FML-low-frequency-group (FML-LF) had different gut microbiome and organic acid profiles. However, the pattern of differences between FML-HF and FML-LF was dissimilar those between LLD and LLnD.

Conclusion

Live LcS can reach the lower gastrointestinal tract alive and may affect the gut microbiome, organic acid profiles and inhibit viral reactivation in the host. These results not only



characterize the impacts of live LcS but will also help to advance research on the maintenance and promotion of health by using the characteristics of LcS.

References

1. Shima T., Kaga C., Shimamoto K., Sugimoto T., Kado Y., Watanabe O., Suwa T., Amamoto R., Tsuji H., Matsumoto S., Characteristics of gut microbiome, organic acid profiles and viral antibody indexes of healthy Japanese with live *Lacticaseibacillus* detected in stool. *Benef Microbes* (2022) 13(1):33-46. doi: 10.3920/BM2021.0101.
2. Shima T., Amamoto R., Kaga C., Kado Y., Sasai T., Watanabe O., Shiinoki J., Iwazaki K., Shigemura H., Tsuji H., Association of life habits and fermented milk intake with stool frequency, defecatory symptoms and intestinal microbiota in healthy Japanese adults. *Benef Microbes* (2019) 10(8):841-854. doi: 10.3920/BM2019.0057.

Presenter was supported by travel grant from Yakult





OP02

Dr. Hannia Leon, ISLI Mesoamerica, Costa Rica

Dr. Hannia Leon.

ILSI Mesoamerica, Costa Rica; executivedirector@ilsimesoamerica.org

ILSI is a global, nonprofit federation dedicated to generating and advancing emerging science and groundbreaking research to ensure foods are safe, nutritious and sustainable, and that they improve planetary and human health and well-being in the 21st century. ILSI Convenes scientists at the forefront of research on nutrition, food safety and sustainability, and operates within a framework of the highest principles of scientific integrity. ILSI's trusted experts and volunteers around the world work synergistically and transparently across academia and the public and private sectors. The aim of this conference is to present the past, present and future of the scientific global portfolio in microbiota, microbiome and healthy aging development by ILSI's entities around the world.

ILSI has ten almost years of experience doing research and capacity building projects about healthy aging and microbiota. ILSI SEA, since 2014 – 2024 generated tree symposium focused in Advancing Gut Microbiome Research – For Application in Health and Food Innovations with a publication calls: Role of the Gut Microbiome in Human Health: A Report of the ILSI Southeast Asia Region Gut Microbiome Conference Series. ILSI Europe has some ongoing projects in the Role of prebiotics in bacterial and viral infection, and vaccination efficiency. Other projects in progress are the development of an inventory of microbiota changes associated with physiological temporal changes in the maternal microbiota before, during and after pregnancy as well as with related clinical observations, and highlight mother and/or infant health benefits and challenges associated with those changes. Some upcoming projects to address the implementation of NAMs in prebiotic research and to identify key markers for assessing microbiota improvement, focusing on measurable indicators of microbiota composition. In Latin America, ILSI Mesoamerica has projects focused on the evaluation of the intestinal microbiota, nutritional and health status of centenarians and their families in Costa Rica blue zone. This project establishes the composition of the intestinal microbiota, identifying the genetic potential of the microbial community in the samples of greatest interest for establishing correlations with the metatypes related to health and nutritional status. In terms of capacity building ILSI has a long history of conferences, symposiums and webinars in topics such as microbiota and healthy ageing, bioactives, probiotics, fibers and microbiota and microbiome. Finally, ILSI has been part of the IUNS – ICN 2017 with a conference about the symbiosis of humans and microbiota and been part of a discussion calls “*Ageing Gracefully: Staying Healthy Late in Life*”

Through its extensive global initiatives, ILSI continues to drive forward the frontiers of microbiota and microbiome research, ensuring that scientific advancements translate into meaningful health and nutrition innovations. By fostering collaboration, supporting cutting-edge research, and promoting knowledge exchange, ILSI remains dedicated to shaping a healthier future for all. As we look ahead, ILSI will continue to expand its scientific portfolio, addressing emerging challenges and opportunities in microbiota, microbiome, and healthy aging—always with the goal of improving human and planetary well-being.

References

Yakult Beneficial Microbes, 27-28 March 2025, Sao Paulo, Brazil



<https://ilsi.eu/scientific-activities/nutrition/healthy-ageing/>;
<https://ilsimesoamerica.org/investigaciones/estudio-de-la-microbiota-intestinal-y-la-situacion-nutricional-de-adultos-de-la-peninsula-de-nicoya-y-del-gran-area-metropolitana-gam/>;
<https://ilsisea-region.org/science-programs/science-clusters/>;
videos here: <https://www.youtube.com/@ILSIGlobal/videos>

Acknowledgement: These research and capacity building projects have been supported by ILSI members from both public and private sectors. More details in ILSI entities websites.

Presenter was supported by travel grant from Yakult Europe



OP03

Probiogenomics: Genomic analysis of probiotics and their health benefits

Vasco Azevedo

Departamento de Genética, Ecologia e Evolução, ICB/UFMG. Av. Antonio Carlos, 6627.
Pampulha, CP 486 CEP 31270-901; Belo Horizonte, Minas Gerais, Brazil.

According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts. For each probiotic strain, safety and efficacy must be evaluated, as well as the analysis of the effects of these microorganisms in ensuring probiosis. In recent years, there has been an advance in sequencing and bioinformatics technologies, which have boosted research in microbiology. The development of in silico analysis platforms, which use omics-based technologies, has allowed these strains to be characterized as potential probiotics, as well as to identify genetic factors, elucidate the complex mechanisms that promote survival, adaptation to the gastrointestinal tract, and facilitate beneficial effects of these microorganisms to the host. Genomic data have enabled a more in-depth analysis of the genetic and molecular factors related to the effects of probiosis, such as antimicrobial and antifungal activities, production of secondary metabolites, genes encoding bioactive compounds such as vitamins, and genes related to immunomodulation and production of anti-inflammatory cytokines. Genomic analyses associated with in vitro and in vivo studies have been used to characterize new probiotic candidates and provide new insights into the main factors associated with these microorganisms' functional and safety characteristics. Through probiogenomic analyses, it is possible to analyze and reveal the molecular basis for the diversity, interaction, and evolution of commensal and probiotic bacteria in promoting host health. This lecture aims to provide an overview of Probiogenomics, its techniques, and its applications, exploring its foundations, advances, and future perspectives.

Presenter was supported by travel grant from FAPESP (2024/21422-5)



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OP04

Microbial therapy of infectious and non-infectious diseases, reasons behind and novel approaches for making probiotics

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Historically fermented products were used by humans for food preservation or as health beneficial supplements. The era of probiotics was developing starting with Metchnikoff studies on *Lactobacillus bulgaricus*. Probiotic industry of making health beneficial bacterial products with LAB or Bifidobacteria constantly grows as multibillion business. However, only recently with appearance of novel data on, metagenomic, metabolomic and proteomic the evidence of health beneficial features of bacteria taken orally became clearer. Now it is evident that these beneficial effects are highly dependent on the host immunity and condition of microbiota. The task of microbial therapy is to drive the microbiota composition from dysbiosis to the balance with the host immune system. In this respect probiotic therapy became more personal and strain dependent. In our studies with probiotic *Enterococcus faecium* strain L3 we demonstrated positive clinical effects in case of different diseases such as multiple sclerosis, IBS and *H. pylori* infection (1,2). However, our recent studies demonstrated the great potential of the indigenous bacteria for restoration of personal microbiota (3). These studies demonstrated that indigenous bacteria (autoprobiotics) taken as mono variants or as consortium of personal bacteria can be beneficial in many pathological conditions of the organism including IBS, HP infection, metabolic syndrome or the cancer (4,5). All these pathologic conditions associated with dysbiosis may be significantly altered by taking indigenous bacteria grown outside the organism as individual probiotics.

References

- Ermolenko, E.I, Gromova L., Borshev, Y., Voeikova A., Karaseva A., Ermolenko K., Gruzdov A., Suvorov A. Influence of Different Probiotic Lactic Acid Bacteria on Microbiota and Metabolism of Rats with Dysbiosis, *Bioscience of Microbiota, Food and Health*, 2013;32(2):41-9. [https://doi: 10.12938/bmfh.32.41](https://doi.org/10.12938/bmfh.32.41).
- Ermolenko E, Rybalchenko O, Borshev Y, Tarasova E, Kramskaya T, Leontieva G, Kotyleva M, Orlova O, Abdurasulova I, Suvorov A. Influence of monostrain and multistain probiotics on immunity, intestinal ultrastructure and microbiota in experimental dysbiosis. *Benef Microbes*. 2018 Dec 7;9(6):937-949. [https://doi: 10.3920/BM2017.0117](https://doi.org/10.3920/BM2017.0117).
- Suvorov A, Karaseva A, Kotyleva M, Kondratenko Y, Lavrenova N, Korobeynikov A, Kozyrev P, Kramskaya T, Leontieva G, Kudryavtsev I, Guo D, Lapidus A, Ermolenko E. Autoprobiotics as an Approach for Restoration of Personalised Microbiota. *Front Microbiol*. 2018 Sep 12;9:1869. [https://doi: 10.3389/fmicb.2018.01869](https://doi.org/10.3389/fmicb.2018.01869)
- Ermolenko E, Kotyleva M, Kotrova A, Tichonov S, Lavrenova N, Voropaeva L, Topalova Y, Karaseva A, Azarov D, Ermolenko K, Druzhininskii D, Dmitriev A, Shishkin A, Suvorov A. Consortium of Indigenous Fecal Bacteria in the Treatment of Metabolic Syndrome. *Microorganisms*. 2022 Aug 5;10(8):1574. doi: 10.3390/microorganisms10081574

Yakult Beneficial Microbes, 27-28 March 2025, Sao Paulo, Brazil



Baryshnikova NV, Ilina AS, Ermolenko EI, Uspenskiy YP, Suvorov AN. Probiotics and autoprobiotics for treatment of *Helicobacter pylori* infection. World J Clin Cases. 2023 Jul 16;11(20):4740-4751. doi: 10.12998/wjcc.v11.i20.4740. PMID: 37583996;

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OP05

Safety evaluations, an essential step in validation of bacteriocin-producing lactic acid bacteria in biotechnological processes

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Research teams routinely isolate bacteriocin-producing microorganisms, contributing to a growing database of new cultures with antimicrobial properties. These bacteriocins have been identified, characterized, and their potential uses in industrial fermentation have been proposed or implemented. Bacteriocins are increasingly recognized for their contribution to the probiotic benefits of numerous microbial cultures. Moreover, antimicrobial peptides from various microorganisms, including lactic acid bacteria (LAB), are viewed as promising alternatives for enhancing food safety and fighting microbial infections in both human and veterinary medicine. Among these, LAB bacteriocins have gained prominence since the discovery of nisin by *Lactococcus lactis*. However, nisin remains one of the most extensively researched bacteriocins and is celebrated for its efficacy and safety as an antimicrobial agent in food preservation.

The number of research projects that are focused upon the isolation and characterization of bacteriocins have continued to escalate and according to www.scopus.com, more than 15.000 research papers have been published concerning different aspects of the production, characterization, and application of bacteriocins. According to the same database more than 85.000 papers have been focused on different aspects of probiotics (accessed on June 2024). The concept of multiple beneficial properties for the safe bacterial culture is a scientific fact, but still not as deeply investigated. Are this can be the next scientific challenge? Characterization of the new beneficial safe cultures, including bacteriocinogenic with aim to be applied as viable microbes in *in situ* biopreservation process include some well-established steps: correct identification, safety evaluation (of producer and antimicrobial agent), characterization of the bacteriocin and suggested application.

Over the past ten years, the search for and evaluation of new microbial strains with multiple advantageous traits have intensified. The goal is to incorporate these multifunctional microbial starters into food fermentation processes, ideally with probiotic or bioprotective qualities in addition to their primary fermentation roles. Safety remains the foremost consideration when selecting new functional microbial cultures. Additionally, the synergy of antimicrobial proteins (bacteriocins) and antioxidants presents an appealing prospect for the development of innovative starter cultures. Such cultures are anticipated to diminish the need for chemical additives, aligning with consumer demands for more natural food products devoid of artificial preservatives. The careful selection of microbial cultures that are safe and possess the desired antimicrobial and antioxidant characteristics marks the initial phase of extensive research

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projects. These projects aim to confirm the technological effectiveness and the proposed health benefits of these cultures through further study.

Bacteriocins from LAB, as members of the broader antimicrobial peptide group, have demonstrated their value in biopreservation. These substances are instrumental in mitigating spoilage, inhibiting foodborne pathogens, prolonging product shelf life, and serving as alternatives to synthetic additives and preservatives. As we delve deeper into the properties and applications of bacteriocin-producing LAB in fermentation and preservation, we uncover more scientific inquiries that require exploration and resolution. A robust scientific foundation currently supports the use of bacteriocins in the food industry and beyond, yet it is imperative to continue this research and development to ensure the provision of food that is safe, nutritious, and beneficial for consumers in the future.

References:

- Choi, G.-H., Holzapfel, W.H., Todorov, S.D. (2023). Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Critical Reviews in Microbiology*. 49(5):578-597. <https://doi.org/10.1080/1040841X.2022.2090227>
- Holzapfel, W.H., Todorov, S.D. (2023). Special issue: Beneficial properties and safety of lactic acid bacteria. Editorial. *Microorganisms*. 11(4):871. <https://doi.org/10.3390/microorganisms11040871>
- Todorov, S.D., Dioso, C.M., Liong, M.-T., Nero, L.A., Khosravi-Darani, K., Ivanova, I.V. (2023). Beneficial features of *Pediococcus*: from starter cultures and inhibitory activities to probiotic benefits. *World Journal of Microbiology and Biotechnology*. 39(1):Article 4. <https://doi.org/10.1007/s11274-022-03419-w>
- Todorov, S.D., Popov, I., Weeks, R., Chikindas, M.L. (2022). The use of bacteriocins/bacteriocinogenic beneficial organisms in food products: benefits, challenges, concerns. *Foods*. 11(19):3145. <https://doi.org/10.3390/foods11193145>

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OP06

Healthy and tasty food: Innovative applications of probiotic lactic acid bacteria in fermented foods

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Functional foods, fortified with bioactive components, have emerged as key players in promoting health and well-being. Among these, fermented foods stand out for their unique blend of taste, texture, and health benefits. In order to develop products that meet the consumers' demands, probiotic lactic acid bacteria are used to produce innovative fermented foods. These bacteria, renowned for their ability to confer health advantages, are pivotal in transforming raw ingredients into nutritionally rich and digestively friendly products.

Probiotic lactic acid bacteria are used to produce a diverse array of fermented foods, including dairy, meat and vegetables products. *Lacticaseibacillus*, *Lactiplantibacillus*, *Levilactobacillus*, *Ligilactobacillus*, *Limosilactobacillus* and *Bifidobacterium* are examples of genera with probiotic strains. Their presence not only enhances flavor and aroma but also delivers a plethora of health benefits. The probiotic lactic acid bacteria can (i) modulate the microbiota; (ii) promote the strengthening of the intestinal barrier; (iii) reduce pathogen competition; (iv) relieve constipation; (v) improve the symptoms of colitis and necrotizing enterocolitis; (vi) contribute to reducing overweight, obesity and blood glucose; (vii) modulate the immune system; (viii) decrease atopic and food allergies; (ix) regulate the central nervous system; (x) alleviate some of the symptoms of autism, depression and anxiety, among others.

Innovations in fermented food production have revolutionized the incorporation of probiotic lactic acid bacteria, ensuring optimal viability and sensorial characteristics. Advanced techniques such as controlled fermentation conditions, strain selection based on functionality, and novel substrate utilization have expanded the repertoire of probiotic-rich products. Precision fermentation technologies enable the production of customized probiotic blends tailored to individual preferences and health needs, paving the way for personalized nutrition. Emerging trends and research in the field of fermented functional foods continue to push the boundaries of innovation. Exploring the role of microbial metabolites in health, scientists are unraveling the intricate interplay between probiotic fermentation and human physiology. Bioinformatics and omics technologies provide insights into microbial communities and their metabolic activities, fueling the discovery of novel probiotic strains and bioactive compounds. In summary, the innovative applications of probiotic lactic acid bacteria in fermented foods represent a nexus of food development and scientific innovation. As consumer demand for functional foods grows, the pursuit of health-promoting fermented products will undoubtedly drive further exploration and discovery in this dynamic field.

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OP07

Bacteriocin production among lactic acid bacteria: A recently discovered probiotic trait having multifarious applications in human and animal health

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Bacteriocins are antimicrobial peptides generally produced by several bacteria including probiotic lactic acid bacteria as part of their defense mechanism. Since long past, these peptides were explored for antimicrobial functions only, but recent landmark discoveries have suggested dynamic functions of bacteriocins in modulation of microbiota, host immunity, anticancer, antibiofilm, antioxidant activity etc. In addition, bacteriocin-producing strains of probiotic bacteria better colonize with higher hydrophobicity as compared to non-producers and show probiotic properties in the host. These peptides have also been proven as an alternative for the treatment of several infectious diseases caused by multi-drug resistant pathogens.

Our group has demonstrated the production of bacteriocins by several lactic acid bacteria isolated from indigenous food and soil samples. A combination of enterocin LD3 and plantaricin LD4 showed synergistic effect against *Staphylococcus aureus* subsp. *aureus* ATCC25923 and *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC13311. The numbers of dead cells were higher as a result of combined effect as compared to their independent effect evidenced by fluorescent microscopy. Transmission electron microscopy revealed the higher disruption of cell membrane in the combined bacteriocin-treated cells as compared to alone effects. The FTIR spectra of enterocin LD3-treated cells showed alteration at $\sim 1,451.82$ and $\sim 1,094.30$ /cm corresponding to nucleic acids and phospholipids suggesting its interaction with cell membrane and nucleic acids. Our data suggest different mode of action of both bacteriocins may be responsible for their synergistic activity against target cells. In addition to antimicrobial activity, these bacteriocins also exhibited anticancer, antioxidant and antibiofilm activity. The producer strains, *Enterococcus hirae* LD3 and *Lactiplantibacillus plantarum* LD4 showed important probiotic attributes such as aggregation, hydrophobicity, bile salt hydrolysis and cholesterol reduction. Thus, bacteriocin producing probiotic bacteria may be used as better probiotic products for human health.

Keywords: Enterocin LD3, Plantaricin LD4, Synergistic effect, *Staphylococcus aureus*, *Salmonella* Typhimurium, Fluorescence microscopy, Transmission electron microscope



OP08

Functional Foods for a Healthy Microbiome

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Probiotics, prebiotics, postbiotics, fermented foods, resistant starch and polyphenols are functional foods, among dietary modifiers containing bioactive molecules with positive effects on gut microbiota composition, diversity and activity, as well as gut integrity, metabolism and modulating the immune system due to the bioactive compounds. Dysbiosis of gut microbiota has been linked to various health problems.

The link between food and the microbiome plays a vital role in health outcomes across various populations, particularly for adults with type 2 diabetes, undernourished children, and women experiencing functional constipation.

Belitung taro (*Xanthosoma sagittifolium*), a low glycemic index and high resistant starch tuber, exhibits prebiotic effects, and promotes the growth of *Butyricimonas*, a butyrate producer. Butyrate is essential for gut health, enhances mucus production and improves insulin sensitivity in type 2 diabetes.

In undernourished children, the probiotic *Lactiplantibacillus plantarum* IS-10506 demonstrates potential in modulating immune responses and nutrient absorption, as shown by significantly increases fecal secretory IgA (sIgA) and serum zinc levels, supported by *in vivo* study of significant higher tight junction (TJ) protein expression, indicating improved gut integrity. In another study, the probiotic strain significantly suppressed allergic reaction as well as atopic dermatitis in adults, by strengthening gut barrier function, and the production of its metabolites as bioactive compounds.

In women with functional constipation, a 21-day supplementation of milk fermented with *Lpb. plantarum* IS-10506 significantly increased relative abundances of Bacteroidetes, particularly *Bacteroides* sp. and *Prevotella* sp. A strong negative correlation between acetate levels and improvements in PAC-Sym scores, alongside significant increases in lactobacilli and Lachnospiraceae, promote eubiosis.

Tempe, an Indonesian soybean-based fermented food is a good plant-based protein source, packed with isoflavone, viable microbes, bioactive peptides, amino acid essential as bioactive compounds, and serves as an antioxidant, and increases diversity of the gut microbiota.

Prebiotic and its effect is successfully established causality between attenuation of diseases and gut microbiota, hence, the potential use of functional foods in precision nutrition is promising. Taken together, taro, probiotic *Lpb. plantarum* IS-10506, fermented foods, postbiotics, and tempe are essential for maintaining gut health and optimizing nutrient absorption across diverse populations.

Keywords: bioactive compounds, functional foods, gut integrity, gut microbiota, mitigate inflammation



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OP09

Dietary microbes, a necessary recommendations for healthy eating?

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In 2001 the “hygiene hypothesis” was put forward again by J-F Bach (1), stating that a contact with *pathogens*, at an early life stage, was necessary to fully mature our immune system. In 2006 (2) this hypothesis was revisited and the importance of the *commensal* microbiota in the training of the immune system of newborns, was put forward (2).

Today more insight has been gained in the importance of a diverse gut microbiota in maintaining health, in offering a higher degree of resilience against possible insults, and in preventing different types of disease.

Microbiota insults, leading to a disturbed, or “dysbiotic”, microbiota, can be manifold, and have been linked to lifestyle factors, such as diet, stress, age, traveling, smoking, or medical interventions by antibiotics, chemotherapeutics, proton pump inhibitors or other drugs. A “dysbiotic” microbiota is increasingly linked to specific health-risk conditions such as obesity, diabetes, allergy or auto-immune diseases, as well as to different types of neural disorders, like anxiety, autism spectrum disorder, Parkinson disease, Alzheimer disease, etc. Currently, however, the crucial cause or consequence question has not often been solved. Correlation does not necessarily mean causation in many diseases, and more in-depth research is needed to solve these links. The problem often is that because of the huge variation in individual microbiota compositions, a “healthy” microbiota is almost impossible to define, making this type of research quite challenging.

Erica and Justin Sonnenburg in 2014 published a paper entitled “Starving our Microbial Self: The Deleterious Consequences of a Diet Deficient in Microbiota-Accessible Carbohydrates” (3) in which they hypothesised that during human evolution we have lost a large part of the microbial diversity in the gut, mostly linked to changes in the diet (change from hunter-gatherer to agriculture, industrial revolution, mass food production, food processing, antibiotic use,...), leading to a reduced intake of fermentable carbohydrates as well as live microorganisms. In the meantime, several, although often rather small studies, have shown that diet indeed is able to positively impact diversity and related immune factors. In order to investigate the hypothesis of ref (3), Wastyck et al (4) could show that the increased intake of fermented foods during a period of 6 weeks had the potential to increase diversity of the gut microbiota as well as to change multiple immune parameters, shifting the immune system towards a more tolerant phenotype. The increased intake of fermentable carbohydrates, however, did not have the same significant effects. This and many other fascinating studies have shown that through diet we can impact on the quality of our microbiota and therefore on maintaining many aspects of health.

References

J-F Bach, 2002. The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases. N Engl J Med 2002; 347:911-920. DOI: 10.1056/NEJMra020100



F. Guarner, R. Bourdet-Sicard, P. Brandtzaeg, *et al.* Mechanisms of Disease: the hygiene hypothesis revisited. *Nat Rev Gastroenterol Hepatol* 3, 275-284 (2006). <https://doi.org/10.1038/ncpgasthep0471>

E. D. Sonnenburg and J. L. Sonnenburg. Starving our Microbial Self: The Deleterious Consequences of a Diet Deficient in Microbiota-Accessible Carbohydrates. *Cell Metab.* 2014 Nov 4; 20(5): 779–786.. doi: 10.1016/j.cmet.2014.07.003; PMID: PMC4896489; PMID: 25156449

H. C. Wastyk, G. K Fragiadakis, D. Perelman, D. Dahan, B. D Merrill, *et al.* 2021. Gut microbiota-targeted diets modulate human immune tatus. *Cell.* 2021 Aug 5; 184(16): 4137–4153.e14. doi: 10.1016/j.cell.2021.06.019. PMID: PMC9020749

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OPI0 Probiotic and probiotic health claims: Comparative regulatory frameworks

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The positive correlation of the increasing concerns about diets consisting of high energy, sugar, salt, aggravated by insufficient physical activity and the increase of diet-related diseases, have become more evident in the last two decades of the 20th century. Likewise, the rising of aged populations observed worldwide correlates with the higher incidence and prevalence of chronic diseases. Both advents highlighted the valuable health effects of what was primarily termed as *Functional Foods* and *Functional Food Products*. Probiotic and Probiotic Foods belong to the scope of *Functional Foods*, whose concept originated in 1984 in Japan, emphasizing the role of FOSHU (Foods for Special Health Uses) foods. Notably, both concepts are older than their definitions. The concept of probiotics as beneficial microorganisms in the intestines being associated with healthier/longer life was described in the book “Prolongation of Life” in the first decade of 1900s, as historically developed by Ilya Ilych Metchnikoff. However, the earliest concept of functional food (FF) dates back to Hippocrates, *ca* 400 B.C: “Let food be your medicine and medicine be your food”. Thus, the primary indicated benefits of functional foods are to decrease and/or to modulate the risk associated with disease. Furthermore, functional foods/ food products may be effective in preventing diseases by generating key physiological/health effects. By the time the above definitions were established, reasonable consensus already highlighted the valuable health effects of what was primarily termed *Functional Foods* and *Functional Food* products. Since then, the market of Functional Foods/Food Products has undergone constant innovation, while novel research frontiers are being developed, mechanisms unveiled, regulations proposed and the relevant terminology continuously redefined. Currently, probiotics, probiotic microorganisms and probiotic food products are among the most studied foods and/or bioactive components, comprising the majority of the food products being regulated and registered as functional. Almost three decades ago Brazil was the sole South American country where regulatory legislation on Functional Foods existed. Similarly to Functional Foods, the terms *probiotic* and *probiotic products* have evolved, and a wide variety of products is available commercially in Brazil and worldwide. Therefore, science-based guidance has become increasingly pivotal to health professionals, industry and consumers, so as to aid the best use of such products as part of balanced diets and healthy lifestyles. In the context of the regulatory framework and guidance having become largely available and increasingly improved, this presentation will focus on the main associative aspects of probiotic food/health regulations/claims in Brazil, as compared to a number of other countries in South America and elsewhere.

References

Belloni A, Charitos IA, Catore S, Topi S, Bottalico S, Santa Croce L. Antibiotics, 2023. Antibiotics 12, 635. <https://doi.org/10.1016/0978-0-12.814625>.

Brasil, Portaria MS/GS no.1549. October 17, 1997. Approved foods for special uses and others. <http://www.anvisa.gov.br/legis/Act/portarias/Index97.htm>. Access July 16th, 2024.

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Brasil, 1997. Portaria MS/GS no.1549. October 17, 1997. Approved foods for special uses and others. <http://www.anvisa.gov.br/legis/Act/portarias/Index97.htm>. Access July 16th, 2024.

Ferreira, CLLF; Bonnet, M. Regulation of Probiotic and Prebiotic Health Claims in South America. In: Sampo Lahtinen; Arthur C. Ouwehand; Seppo Salminen; Atte von Wright. (Org.). Lactic Acid Bacteria. 4ed. Florida: CRC Press, Taylor and Francis Group, 2012, p. 749-759.

Hawkes, C. 2004. Nutrition labels and health claims: The global regulatory environment. WHO (2004), Geneva. Access July 16th, 2024.

Hirahara, T. 2004. Key factors for the success of Functional Foods. The Proceedings of the 3rd International Conference on Food Factors (ICOFFO3). <https://content.iospress.com/articles/biofactors/bio00598>. Access July 16th, 2024.

Metchnikoff, I.I. The prolongation of life: optimistic studies. Publication/Creation, London: William Heinemann, 1907. Access June, 2016.

Soichi, A. Functional Food Science in Japan: State of the art. Biofactors 12 (2000)13-15 IOS Press. Access June, 2024.

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OPII

Probiotics for Safe and Healthy foods: Embrapa's Program

“One Health”

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The concept of “One Health” highlights the important interrelationships between health and well-being of people, animals, plants, and the environment which supports their existence. The One Health High Level Expert Panel (OHHLEP) has redefined One Health in 2021 by stressing an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. One Health refers to a systemic agenda that countries should implement in an integrated way. EMBRAPA – Brazilian Agriculture Research Corporation, a public corporation under the Brazilian Ministry of Agriculture, focusing on scientific and technological research for agriculture in Brazil, has been building a One Health R&D Program since 2023. It aims at structuring high-level, multicentric, transdisciplinary and collaborative R&D projects considering the key pillars of One Health. Primary program strategies include not only the development of advanced descriptive, prescriptive and predictive system capacities, early detection and identification of hazards and associated risks, rapid response and systemic surveillance, but also the strategic use of biotic and abiotic assets for global health benefit. In fact, one of the action tracks of the Program focuses on “Strengthening the assessment, management and communication of food safety risks”. The safety of food is linked inextricably to where it is grown (environment), how it is grown (human-animal-plant-environment interactions) and also how it is consumed (human interactions). As a central component of One Health, dietary habits emerge as an important modifiable factor for health benefits. Beyond satisfying nutritional requirements, diet influences the composition and metabolic activity of the microbial ecosystem in the gut. Probiotic microorganisms, ingested regularly in adequate amounts, can reach the colon and contribute beneficially to the gut microbiome, which includes the promotion of mucosal immunity, enhancement of intestinal epithelial barrier and modulation of systemic metabolic responses. Since the survival of probiotics depends on the food matrix, strategies to improve survivability and adequate delivery to the consumer have achieved important advances. The use of probiotic microorganisms and/or their antimicrobial compounds to improve safety and extend shelf life through biopreservation requires minimal process interventions, while contributing to consumer's health. Current initiatives developed by EMBRAPA and partners include prospecting autochthonous bacteria that are beneficial to health, whether due to their intrinsic probiotic potential or the production of substances such as enzymes and antimicrobials; incorporating probiotics directly into food matrices, or using techniques such as encapsulation to promote the extension of their viability and controlled release; isolation, characterization, evaluation of probiotic activity; development of novel dairy and non-dairy products as carriers of probiotic microorganisms; and hurdle technologies for food preservation. Collaboration with institutions and companies aiming at developing novel processes and products to promote health and prevent diseases under the One Health rationale has become an increasing key driver for food security and safety in Brazil and worldwide.

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References:

Touseef Ahmed, Muhammad Farooq Tahir, Lisa Boden and Tigga Kingston. Future directions for One Health research: Regional and sectoral gaps. *One Health* 17 (2023) 100584

ONE HEALTH HIGH-LEVEL EXPERT PANEL. The One Health definition and principles developed by OHHLEP: translations. [Geneva]: WHO, 2023. 9 p. Disponível em: <https://www.who.int/publications/m/item/one-health-definitions-and-principles>. Acesso em: 27 set. 2023.

Amauri Rosenthal, Andréa Madalena Maciel Guedes, Karina Maria Olbrich dos Santos, Rosires Deliza, Healthy food innovation in sustainable food system 4.0: integration of entrepreneurship, research, and education, *Current Opinion in Food Science*, Volume 42, 2021, Pages 215-223, ISSN 2214-7993, <https://doi.org/10.1016/j.cofs.2021.07.002>.



OPI2

Gut microbiota development and short-chain fatty acid composition in early life

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Introduction

In early life, the gut microbiome plays a crucial role in shaping the long-term health outcomes. Among the various factors involved, short-chain fatty acids (SCFA) produced by gut bacteria have emerged as significant mediators of complex host-microbiota interactions. However, our understanding of the specific SCFAs present in the infant gut, their quantitative profiles, developmental changes, and precise contribution of gut microorganisms to SCFA production remains limited. This study aimed to conduct a comprehensive examination of the gut microbiota and SCFA composition during infancy, with the objective of elucidating their relationships.

Method

Stool samples were obtained from 12 healthy individuals during their initial two years of life. A comprehensive analysis of 1,048 stool samples (averaging 87 per subject) was conducted to examine bacterial composition through meta-16S analysis and to quantify SCFA concentrations utilizing high-performance liquid chromatography (HPLC).

Result 1: Infant-type Bifidobacteriales correlated with gut lactate and formate concentrations.

Infant gut microbiota development began with Enterobacterales dominance, gradually shifting to Bifidobacteriales and Clostridiales. During the first two years, infant gut SCFA composition differed from adults, with higher formate and lactate levels. Although these SCFA correlated with Bifidobacteriales abundance, their increases did not always coincide. Generally, Bifidobacteriales abundance rose before lactate and formate levels. Species-level analysis showed a temporal relationship between the growth of bifidobacteria utilizing fucosylated Human Milk Oligosaccharides (specifically *Bifidobacterium infantis*, *Bifidobacterium bifidum*, and *Bifidobacterium breve*) and increased gut SCFA concentrations.

Result 2: Diverse and personalized Clostridiales species were involved in butyrate production after breastfeeding cessation.

Correlation analysis between gut microbiota composition and SCFA concentration revealed that Clostridiales and butyrate were highly correlated, and both increased at the breastfeeding cessation. Then, we investigated the presence or absence of a butyrate-producing pathway in Clostridiales and found that butyrate-producers were part of the Clostridiales present in the infant's gut and also consisted of diverse and personalized Clostridiales species.

Conclusion

In this study, the dense time series sampling from infants allowed for a detailed analysis of the relationship between infant gut microbiota and SCFA. SCFA has been reported to be associated



with health. The results of this study are expected to help to develop methods to control gut microbiota, SCFA concentrations during infancy, and ultimately protect their future health.

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References

1. Tsukuda N, Yahagi K, Hara T, Watanabe Y, Matsumoto H, Mori H, Higashi K, Tsuji H, Matsumoto S, Kurokawa K, Matsuki T. 2021. Key bacterial taxa and metabolic pathways affecting gut short-chain fatty acid profiles in early life. *ISME J* 15: 2574-2590.
2. Matsuki T, Yahagi K, Mori H, Matsumoto H, Hara T, Tajima S, Ogawa E, Kodama H, Yamamoto K, Yamada T, Matsumoto S, Kurokawa K. 2016. A key genetic factor for fucosyllactose utilization affects infant gut microbiota development. *Nat Commun* 7: 11939.

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OP13

Microbiota modulation and sports performance

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The gut microbiota is a complex community with countless correlations with health status, well-being and even athletic performance. In this study, we investigated the changes in the gut microbiota of runners participating in the Brazil 135 Ultramarathon. We also wanted to evaluate the production of specific metabolites in the gut as well as the modulation and resilience as a consequence of the 217 km race. The study was approved by the local Research Ethics Committee (n. 4.179.685; July 29, 2020). GM composition was determined by real-time PCR analysis and shotgun metagenomic sequencing. Taxonomic profiling of the samples was performed using MetaPhlAn 3. The Shannon and Simpson index was calculated using the `alpha_diversity.py` script from the Qiime2 pipeline (http://qiime.org/scripts/alpha_diversity.html). Short-chain fatty acids were analyzed with HPLC and fecal ammonia with a specific ion analyzer. Our research team followed ultramarathon athletes and observed significant fluctuations in the proportion of certain microbial taxa and metabolites from volunteers' stool samples. These fluctuations may be related to performance during the race. This research may aid in the development of therapeutic and dietary strategies to modulate the composition of gut microbiota for these fins. These results may also help to clarify how physical activity, and its variables (modality, intensity and duration) may influence the composition of the gut microbiota. In addition, our team has developed a probiotic sports beverage. We investigated the effect of fermented milk enriched with whey protein (approx. 80 % protein), probiotic (*Bifidobacterium animalis* BB12) and pomegranate juice (*Punica granatum* L.) on physical performance, intestinal motility and villus structure, inflammatory markers and gut microbiota of rats under high-intensity acute exercise. A total of twenty-four Wistar rats were divided into groups: Control (Ctrl), Supplemented (Supp), Exercised (Exe) and Exercised and Supplemented (Exe+Supp). The rats in the Supp groups received fermented milk by oral administration for 6 weeks. This work was approved by the Animal Use Ethics Committee (CEUA) of University of Campinas (protocol 4150-1) and was conducted under the ethics standards for animal experimentation of the Brazilian College of Animal Experimentation (COBEA). At the end of the supplementation period, the



Exe groups were subjected to high-intensity acute exercise on a treadmill. We found that intense acute exercise caused changes in the gut villus interstitial space, changes in the proportion of *Lactobacillus* species and an increase in *Clostridium* species, as well as a decrease in gut motility. Dietary supplementation increased intestinal motility and maintained the inter-intestinal villus space and the natural proportions of the microbiota of the trained rats. New studies may elucidate specific mechanisms and help with personalized recommendations aimed at improving performance and/or recovery.

References

CHAVES, F. M. ; BATISTA, I. L. ; SIMABUCO, F. M. ; QUARESMA, P. G. F. ; BEZERRA, R. M. N. ; PAULI, J. R. ; CUNHA, D. T. ; FERRAZ, P. L. C. ; ANTUNES, A. E. C. . High-intensity exercise-induced intestinal damage which is protected by fermented milk added by whey protein, probiotic and pomegranate (*Punica granatum* L.). BRITISH JOURNAL OF NUTRITION, v. 119, p. 896-909, 2018. <https://doi.org/10.1017/S0007114518000594>.

SARAGIOTTO, GIULIO KAI ; DE OLIVEIRA, LUIZ FELIPE VALTER ; DE OLIVEIRA, MILENA MERIZZI ; Simabuco, Fernando Moreira ; BELLI, TAISA ; ANTUNES, A. E. C. Does a 217-km mountain ultramarathon affect the gut microbiota of a top 10 runner at the Brazil 135 Ultramarathon?. SCANDINAVIAN JOURNAL OF MEDICINE & SCIENCE IN SPORTS, p. 1-3, 2023. <https://doi.org/10.1111/sms.14512>

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OPI4 Experience of Autoprobiotics Application for Correction Human Microbiota Under Conditions of Isolation and Dry Immersion

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Artificially modified habitat is created when it is needed to learn the nature of things that lie outside the framework of everyday conditions of existence. This, first of all, concerns the exploration of space, the ocean and earth underground resources. To help, artificial anthropo-ecosystems are created with modified habitat parameters. To correct dysbiosis, which is one of the main risk factors while this activity, a wide arsenal of probiotics based on collection strains of microorganisms - representatives of protective groups - is widely used. One of the directions of modern prevention and therapy of dysbacteriosis is the application of autoprobiotics. It seems that autoprobiotics, as part of a complex of personalized prevention agents, can be an active factor in counteracting the development of the syndrome of colonization resistance decrease, primarily the first barrier to colonization - autochthonous protective microflora - in humans in modified environment. We investigated effectiveness of autoprobiotics on microbiota content of volunteers in experiments, simulating 2 spaceflight factors – long-term isolation, simulated weightlessness and on microflora of primates under modified radiation.

Objectives:

1. To investigate the effectiveness of the impact of autoprobiotics on the quantitative and generic composition of microflora of primates subjected to modified radiation.
2. To investigate the effectiveness of the impact of autoprobiotics on the quantitative and generic composition of microflora of volunteers in experiments with long-term isolation.
3. To investigate the activity of autoprobiotics on the quantitative and generic composition of microflora volunteers in “dry immersion” experiments.

The stabilizing effect of autoprobiotics on the quantitative and species characteristics of intestinal microflora, microflora of the upper respiratory tract was demonstrated in experiments involving humans, simulating the impact of such space flight factors as long-term isolation in confined object and under simulated microgravity (the “dry immersion” experiment), Comparative studies of the intestinal microflora in humans were carried out during a period of long-term isolation without taking prophylactic agents, with taking prophylactic agents based on probiotics, made on the basis of collection cultures and autoprobiotics. The use of autoprobiotic preparations in experiments simulating the impact of space flight factors on the human body (isolation, dry immersion) and animals (irradiation) makes it possible to optimize the quantitative and species composition of microflora, thus preventing the risk of the formation of auto- and cross-infections during the period of acute adaptation. The use of autoprobiotics leads to persistent stabilization of protective microflora and a quantitative reduction of opportunistic microflora with a long-term - up to 30 days - potentiated effect.

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Yakult Beneficial Microbes, 27-28 March 2025, Sao Paulo, Brazil





OP15 Modulatory effect of Daily Intake of Fermented Milk Containing *Lactocaseibacillus paracasei* cepa Shirota (LcS) on Upper Airways Immune/Inflammatory Responses in Marathon Runners

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Background

Although *Lactocaseibacillus paracasei* cepa Shirota (LcS) can benefit the immune status, the effects of LcS on the immune/inflammatory responses in the upper airways of marathon runners have never been evaluated. Therefore, here we evaluated the effect of daily ingestion of fermented milk containing or not LcS in the upper airway immune/inflammatory responses before and after a marathon.

Methods

Forty-two male marathon runners ingested a fermented milk containing 40 billion of LcS/day (LcS group, n=20) or placebo (unfermented milk, n=22) for 30 days pre-marathon. Immune/inflammatory parameters, such as neutrophil infiltration on nasal mucosa and also the concentrations of secretory IgA (SIgA), cytokines, and antimicrobial peptides in saliva, were evaluated before and after fermented milk ingestion, immediately, 72h, and 14d post-marathon.

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Results

Higher proinflammatory cytokine levels and also lower levels of SIgA and antimicrobial peptides, were found immediately post-marathon in the placebo group compared to other time points and in the LcS group. On the other hand, higher anti-inflammatory cytokine levels and reduced neutrophil infiltration on nasal mucosa were found in the LcS group compared to other time points and in the placebo group.

Conclusion

Our findings showed, for the first time, that the daily ingestion of LcS pre-competition was able to modulate the upper airway immune/inflammatory responses post-marathon.

Keywords: antimicrobial peptides; cytokines; neutrophil infiltration; probiotics; secretory immunoglobulin A.

References

- Baken K.A., Ezendam J., Gremmer E.R., de Klerk A., Pennings J.L., Matthee B., Peijnenburg A.A., van Loveren H. Evaluation of immunomodulation by *Lactobacillus casei* Shirota: immune function, autoimmunity and gene expression. *Int. J. Food Microbiol.* 2006;112:8–18. doi: 10.1016/j.ijfoodmicro.2006.06.009.
- Gleeson M., Bishop N.C., Oliveira M., Tauler P. Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. *Int. J. Sport Nutr. Exerc. Metab.* 2011;21:55–64. doi: 10.1123/ijsnem.21.1.55.
- Shida K., Nanno M., Nagata S. Flexible cytokine production by macrophages and T cells in response to probiotic bacteria: a possible mechanism by which probiotics exert multifunctional immune regulatory activities. *Gut Microbes.* 2011;2:109–114. doi: 10.4161/gmic.2.2.15661.

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OPI6 Discovering functional probiotics protecting against inflammatory bowel disease

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Inflammatory bowel disease (IBD), which is comprised of Crohn's disease (CD) and ulcerative colitis (UC), is characterized by chronic inflammation associated with intestinal immune dysfunction and disruption of intestinal barrier integrity. Recent studies and clinical trials have shown that probiotics could be used as a novel therapeutic agent to relieve gut inflammation and to extend remission. However, since probiotic strains are known to be involved highly complex interaction with commensal microbes and/or the host, demystifying underlying mechanism of beneficial probiotics is still a huge challenge. In this study, potential probiotic strains were screened and selected based on their safety and functionality, and those selected strains were subjected to deeper investigation to identify microbiota-mediated effect as well as host-directed effect.

Potential probiotic isolates were provided by HEM Pharma, tested *in vitro* for safety following EFSA guideline (2018), and screened for immunomodulatory function using murine macrophage cell line (Raw264.7). For animal study, specific pathogen free (SPF) C57BL/6J female mice were administered with each probiotic strain at 1×10^9 CFU/mouse/day for 3 weeks, treated with 3% DSS for 7 days and drinking water for 2 days. Interleukin (IL)-10 knockout female mice were treated with each strain at 1×10^8 CFU/mouse/day and 1.5% DSS. Germ-free mice were bred in sterile isolator, transported to individually ventilated cage, and then mono-colonized with each strain by feeding 5×10^8 CFU/mouse. After 2 weeks, mice were treated with 1% DSS for 7 days. All animal experiments were approved by the Committee on the Ethics of Animal Experiments of the Handong Global University. mRNA expressions were analyzed by real-time RT-PCR, and protein expression levels were measured by ELISA. For microbiota analysis, fecal or cecal DNA was extracted, amplified using 16S rDNA V3-V4 primers, barcoded, purified and sequenced on a MiSeq Illumina platform. OTUs were picked on the Greengenes database and further analyzed using QIIME 2 pipeline. Metabolites were extracted and quantified by gas chromatography-mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS).

Based on safety, immunomodulatory function tests using Raw264.7 and efficacy tests using the DSS-induced colitis model, *Limosilactobacillus fermentum* 858 and *Latilactobacillus curvatus* 391 were selected as potential probiotics against colitis. Administration of *Limosilactobacillus fermentum* 858 or *Latilactobacillus curvatus* 391 ameliorated DSS-induced body weight loss, colon length shortening, histopathological features, and modulated expressions of pro-inflammatory cytokines and intestinal tight junction proteins. Moreover, the inducible regulatory T cell population (Nrp-1⁺Helios⁺Foxp3⁺) in colon tissue was significantly increased in the *Limosilactobacillus fermentum* 858-treated group. The treatment of those



strains also alleviated DSS-induced gut microbiota dysbiosis and changed metabolite composition. Interestingly, the improving effect of *Limosilactobacillus fermentum* 858 was substantially diminished in IL-10 knockout mice, while *Latilactobacillus curvatus* 391 was still effective, suggesting that *Limosilactobacillus fermentum* 858 protects against colitis in an IL-10-dependent way. Meanwhile, mice mono-colonized with each strain showed enhanced prevention against DSS-induced colitis and modulated metabolite composition. In the humanized mouse model of fecal microbiota transplantation, *Limosilactobacillus fermentum* 858 supplementation increased colonic IL-10 production in parallel with an augmentation of body weight gain and colon length. The treatment of EPS extract isolated from *Limosilactobacillus fermentum* 858 and *Latilactobacillus curvatus* 391 augmented IL-10 production in murine splenocytes. Taken together, the protection of *Limosilactobacillus fermentum* 858 and *Latilactobacillus curvatus* 391 against colitis would be a comprehensive effect mediated by both microbiota-dependent and host-directed mechanisms.

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Keywords: Inflammatory Bowel disease; DSS-induced colitis; Mono-colonization; metabolomics; metagenomics



OPI7

Microbiome and Atherosclerosis Biomarkers or How could we prevent age- and stress- relevant diseases individually

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In recent years, the role of intestinal and oral microbiomes in human mental health is defined. Dutch and British scientists found a link between the bacterial composition of the intestines and depression in representatives of different ethnicities [1, 2]. Gut microbiome samples were compared with results of depression tests of 2,593 volunteers and the association of bacterial compositions and mental disorders were confirmed. Thirteen microbial taxa were most important, including *Eggerthella*, *Subdoligranulum*, *Coprococcus*, *Sellimonas*, *Lachnoclostridium*, *Hungatella*, *Ruminococcaceae*, *Lachnospiraceae* and *Eubacterium ventriosum*. Among these, *Hungatella*, *Sellimonas*, *Eggerthella* and *Lachnoclostridium* were more abundant in people with higher depressive symptoms, while the rest were less abundant. Many bacteria are known to be involved in the synthesis of neurotransmitters such as serotonin, glutamate, gamma-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT) and catecholamines, which play important roles in brain and mental health. Disturbance of their normal ratio in the gut changes the concentrations of these neurotransmitters in the body and affects the brain in a way that leads to depression. Some bacterial strains that affect the 5-HT pathway and regulate catecholamines are included in health-promoting functional foods that have significant effects on the regulation of neurometabolites [3]. Israeli scientists recently isolated the same signaling microorganisms from the saliva of all 200 examined veterans with posttraumatic stress disorders, PTSD [4]. Expression of signaling microbiota (i.e., decreased levels of sp_HMT_914, 332, and 871 and *Noxia* bacteria) was correlated with PTSD severity, for example, with obsessions, agitation, and reactivity, as well as additional psychopathological symptoms, including anxiety, hostility, memory problems and idiopathic pain. In contrast, duration of treatment correlated with significantly increased levels of sp_HMT_871 and decreased levels of *Bacteroidetes* and *Firmicutes* and was inversely correlated with adverse psychopathology scores.

We discovered a) unique markers of chronic inflammation and vascular elasticity (an increased number of *Oscillospiraceae* UCG-002, *Fusicatenibacter*, *Erysipelotrichaceae* UCG-003,



Christensenellaceae R-7 group, *Monoglobus*, *Muribaculaceae*, *Coriobacteriales*, *Anaerovoracaceae*, *Eggerthellaceae*) and b) new biomarkers of early symptoms of these disorders (*Veillonellaceae*, *Muribaculaceae*, *archaea* WPS-2, *Succinivibrio*, *Elusimicrobium*, *Akkermansiaceae*, *Bacteroides*). The expression of genes regulating lymphocyte immunometabolism in patients with COVID-19 and T2DM was determined, depending on the type of hypoglycemic therapy. Correlations were determined between the expression levels of PRKAA1, SLC2A1, MTOR and biomarkers of inflammation (procalcitonin, C-reactive protein, neutrophil-lymphocyte ratio). Correction of the microbiome by various biotics is proposed on the principles of 3P medicine [5]. Our algorithm and IS developed and widely reported were applied for targeted correction of different microbiome compositions specific to “diseases” stage based on individual omics profile by individual nutrition, physical and rehabilitative psychological activity recommendation, relay on life style, pollution etc. Bioactive compounds of the unique technology of extraction, postbiotics and psychobiotics developed on unique strains of probiotic microbes and their metabolites, which are capable to personalized prognostic correction of an individual intestinal and oral microbiota, and therefore biochemical and mental health for prevention/treatment of post-traumatic stress syndrome and age-stress-related diseases are promising medication tools and currently under clinical investigation.

References

1. Baião, R., Capitão, L.P., Higgins, C., Browning, M., Harmer, C.J., Burnet, P.W.J., 2022. Multispecies probiotic administration reduces emotional salience and improves mood in subjects with moderate depression: a randomised, double-blind, placebo-controlled study. *Psychol. Med.* 1–11. <https://doi.org/10.1017/S003329172100550X>
2. Bosch, J.A., Nieuwdorp, M., Zwinderman, A.H., Deschasaux, M., Radjabzadeh, D., Kraaij, R., Davids, M., de Rooij, S.R., Lok, A., 2022. The gut microbiota and depressive symptoms across ethnic groups. *Nat. Commun.* 13, 7129. <https://doi.org/10.1038/s41467-022-34504-1>
3. Chudzik, A., Orzyłowska, A., Rola, R., Stanisz, G.J., 2021. Probiotics, Prebiotics and Postbiotics on Mitigation of Depression Symptoms: Modulation of the Brain–Gut–Microbiome Axis. *Biomolecules* 11, 1000. <https://doi.org/10.3390/biom11071000>
4. Levert-Levitt, E., Shapira, G., Sragovich, S., Shomron, N., Lam, J.C.K., Li VoK, Heimesaat, M.M., Bereswill, S., Yehuda, A.B., Sagi-Schwartz, A., Solomon, Z., Gozes, I. Oral microbiota signatures in post-traumatic stress disorder (PTSD) veterans. *Mol Psychiatry.* 2022 Nov;27(11):4590-4598. doi: 10.1038/s41380-022-01704-6. Epub 2022 Jul 22. PMID: 35864319.
5. Microbiome in 3P Medicine Strategies. (2023). In N. Boyko & O. Golubnitschaja (Eds.), *Advances in Predictive, Preventive and Personalised Medicine*. Springer International Publishing. <https://doi.org/10.1007/978-3-031-19564-8>

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OP18

The role of the microbiota-immune system axis in probiotic functions

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The intestinal microbiota comprises trillions of microorganisms (bacteria, viruses, fungi) that inhabit the human gastrointestinal tract, from the mouth to the large intestine. Bacteria comprise the majority of this ecosystem and perform important functions for our body, including saccharolytic and proteolytic fermentation, B and K vitamins' synthesis, induction of mucus and antimicrobial peptides' secretion, protection against pathogenic microorganisms by nutrient competition, and maturation of the mucosal immune system. Several factors can impact the initial colonization of the newborn's gut, including the maternal microbiota status, mode of birth, breastfeeding or formula, antibiotics, genetic and environmental factors. The establishment of the microbiota soon after birth plays a fundamental role in the maturation of the mucosal immune system, producing metabolites and providing essential signals for the education of immune cells and influencing the proper functioning of our immune system in adult life. The imbalance of the intestinal microbiota early in life, a process known as dysbiosis, can impact the immune response in infectious diseases, and favor the development of some diseases in later life, including allergies and obesity. In this context, probiotics present two different immunomodulatory impacts on the host and can induce a anti- or pro--inflammatory immune responses. In an anti-inflammatory response, some probiotic strains can induce regulatory T cells, *via* dendritic cell modulation in the gut mucosa, IL-10 and TGF- β induction, enhancing the IgA production and gut barrier function. In an immunostimulatory response, an increased phagocytosis and activity of macrophages, dendritic cells, and neutrophils were observed, as well as, increased NK cell activity, inflammatory cytokines' secretion, and Th1/Th17 polarization in the gut mucosa. Therefore, knowledge of the probiotic strain and experimental studies are essential to determine the best strain to achieve the therapeutic aims.

References

1. Oliveira GLV, Oliveira CNS, Pinzan CF, de Salis LVV, Cardoso CRB. Microbiota Modulation of the Gut-Lung Axis in COVID-19. *Front Immunol.* 2021 Feb 24;12:635471. doi: 10.3389/fimmu.2021.635471.
2. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics Mechanism of Action on Immune Cells and Beneficial Effects on Human Health. *Cells.* 2023 Jan 2;12(1):184. doi: 10.3390/cells12010184.
3. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-Inflammatory and Immunomodulatory Effects of Probiotics in Gut Inflammation: A Door to the Body. *Front Immunol.* 2021 Feb 26;12:578386. doi: 10.3389/fimmu.2021.578386.
4. Donald K, Finlay BB. Early-life interactions between the microbiota and immune system: impact on immune system development and atopic disease. *Nat Rev Immunol.* 2023 Nov;23(11):735-748. doi: 10.1038/s41577-023-00874-w.



OP19

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The role of probiotics and prebiotics in inducing gut immunity

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Current estimates on the number of people who practice street running in the world is about 620 million people. In Brazil, the calculation is about 12 to 15 million runners. The fact that 8% of the world's population and 5% of the Brazilian population practice this form of exercise, which is cheap and accessible, is very positive. However, we must remember that, according to the WHO, more than 90% of the planet's population lives in polluted environments, which raises the question of health, not only of runners, but of the population in general.

About two decades ago, the great topic under debate among researchers in the field of sports medicine was that after exhausting exercises, it was very common for the person to have a flu-like condition.

The predominant explanation at the time was that the individual had a period of systemic immunosuppression, called the "open window theory". However, this explanation seemed strange to me since successive periods of immunosuppression did not have repercussions on other systems.

Taking this occurrence as a research problem, Prof. André Bachi and I studied the mucosal immune response, which allowed us to verify that being the response of the systemic immune system, distinct from the mucosal immune response, pointed us to an alternative explanation for the finding of the flu-like condition of the athletes.

Next, our group studied the airway response of runners, comparing them with sedentary runners, in the face of pollution, and found that exercise generated a protective effect against pollution, both at the level of local immune response and systemic response. This finding is probably due to the anti-inflammatory effect determined by regular and moderate exercise practice.

Our next step was to study the effect of different levels of pollution on mice placed in a pollution concentrator. In it, sedentary mice and runners were exposed to environmental pollution and pollution levels at which 2.5 particulate matter was concentrated 20 times.

In accordance with data in the literature, we were able to verify that only mice that ran in an environment of extreme pollution presented a systemic inflammatory response. We were also able to observe, both in the study of serum cytokines and bronchoalveolar lavage, that there was a strongly positive correlation between pro- and anti-inflammatory cytokines, suggesting that the organism would be seeking a balance.

In partnership with Prof. André Bachi, we studied, at that time, the airway of marathon runners using LcS or placebo. We were able to verify that, even before the race, the number of neutrophils infiltrated in the nasal mucosa of the LcS group was statistically lower than the placebo group.

This finding drew our attention to the protective action of probiotics on pollution, which is still and likely to be a major public health problem for decades to come. Most of the literature shows

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the beneficial effects of probiotics, in relation to the harms of pollution, which we will discuss during the presentation. However, practically all of them, study only the systemic response, which in our view leaves a gap related to the mucosal immune response.

Finally, addressing the proposed mechanisms of action of probiotics, as well as the mechanisms by which the probiotic has a protective effect against pollution, will allow us to focus on the gut-lung axis, the means by which the healthy gut microbiota contributes to lung and airway homeostasis.



OP20

Sporeforming probiotics for poultry: what, where, and how?

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Although many countries have officially banned the use of antibiotics as a preventive measure for control of common infections and promoting growth in livestock, some farmers still use them. This persistent practice raises concerns about the development of antibiotic-resistant bacteria, posing significant risks to public health and food safety.

Consumer demand for antibiotic-free animal products has become a very strong incentive for official regulatory agencies and the agricultural industry to use probiotics in livestock, and poultry in particular. The shift towards antibiotic-free production systems has encouraged the exploration of probiotics as a natural and effective strategy to enhance animal health, productivity, and disease resistance. Similar to human probiotics, many of those utilized in poultry belong to lactic acid bacteria. However, there is growing interest in *Bacillus* spp. sporeforming probiotics due to their wide range of health benefits, biotechnological advantages, stability during production and long (compared to non-sporeforming probiotics) shelf life, ease of incorporation into animal feed, and, last but not least, cost-effectiveness [1]. Additionally, *Bacillus* spp. are known for their resilience in harsh environmental conditions, including extreme temperatures, desiccation, and pH variations, which further enhances their applicability in diverse agricultural settings. Their ability to form endospores makes them particularly advantageous for commercial feed formulations, ensuring their viability during storage and transport.

We reported the ability of probiotic strains *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895 to promote the poultry's growth without increasing feed consumption while also protecting against pathogens [2]. These strains have been shown to improve key productivity indicators, including live weight gain, egg production, and reproductive performance in both hens and roosters. Notably, their supplementation resulted in higher body weights compared to control groups, with significant improvements observed in the development of internal organs such as the heart, liver, and reproductive organs.

In addition, spore-forming probiotics demonstrated *in vitro* SOS-inhibitory activity and the ability to reduce SOS-associated mutagenesis in bacteria [3], which is critical in mitigating the risk of antibiotic resistance development. By suppressing the bacterial SOS response, these probiotics may help limit the horizontal transfer of antibiotic-resistance genes, contributing to antimicrobial stewardship efforts. They also modulate the immune system of the birds [4], enhancing both innate and adaptive immune responses, which potentially leads to increased resistance to infectious diseases and improved vaccine efficacy.



Interestingly, in *in vivo* studies, the health benefits of some sporeforming bacilli derived from poultry were greater than those of other origins [4], suggesting a host-specific adaptation that optimizes probiotic efficacy. This host specificity underscores the importance of selecting strains that are naturally compatible with the target species to maximize health benefits and ensure consistent performance under commercial farming conditions.

The multiple applications of these probiotics and the benefits of their biotechnological production using the solid-state fermentation process as a key component of sustainable agriculture aimed at the valorization of agricultural bio-waste will also be discussed [3, 5].

References

1. Todorov, S.D.; Ivanova, I.V.; Popov, I.; Weeks, R.; Chikindas, M.L. *Bacillus* spore-forming probiotics: benefits with concerns? *Crit. Rev. Microbiol.* 2022, 48(4), 513-530. <https://doi.org/10.1080/1040841X.2021.1983517>
2. Prazdnova, E.V.; Mazanko, M.S.; Chistyakov, V.A.; Denisenko, Y.V.; Makarenko, M.S.; Usatov, A.V.; Bren, A.B.; Tutelyan, A.V.; Komarova, Z.B.; Gorlov, I.F.; Weeks, R.; Chikindas, M.L. Effect of *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens* B-1895 on the productivity, reproductive aging, and physiological characteristics of hens and roosters. *Benef. Microbes.* 2019, 10(4), 395-412. <https://doi.org/10.3920/BM2018.0149>
3. Prazdnova, E.V.; Mazanko, M.S.; Bren, A.B.; Chistyakov, V.A.; Weeks, R.; Chikindas, M.L. SOS response inhibitory properties by potential probiotic formulations of *Bacillus amyloliquefaciens* B-1895 and *Bacillus subtilis* KATMIRA1933 obtained by solid-state fermentation. *Curr. Microbiol.* 2019, 76(3), 312-319. <https://doi.org/10.1007/s00284-018-01623-2>.
4. Mazanko, M.S.; Popov, I.V.; Prazdnova, E.V.; Refeld, A.G.; Bren, A.B.; Zelenkova, G.A.; Chistyakov, V.A.; Algburi, A.; Weeks, R.M.; Ermakov, A.M.; Chikindas, M.L. Beneficial effects of spore-forming *Bacillus* probiotic bacteria isolated from poultry microbiota on broilers' health, growth performance, and immune system. *Front. Vet. Sci.* 2022, 9, 877360. <https://doi.org/10.3389/fvets.2022.877360>
5. Mahoney, R.; Weeks, R.; Huang, Q.; Dai, W.; Cao, Y.; Liu, G.; Guo, Y.; Chistyakov, V.A.; Ermakov, A.M.; Rudoy, D.; Bren, A.; Popov, I.; Chikindas, M.L. Fermented duckweed as a potential feed additive with poultry beneficial bacilli probiotics. *Probiotics Antimicrob. Proteins.* 2021, 13(5), 1425-1432. <https://doi.org/10.1007/s12602-021-09794-4>

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OP21

Intensified but sustainable aquaculture, the implication of novel microbial feed supplement

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There is increasing demand for fish and shellfish as a promising source of protein for the increasing world population. Also, there is more focus on sustainable and environment-friendly aquaculture practices, known as "blue farming." Therefore, there may be a rising demand for feed additives to support these goals. A common approach for increasing the production rate to meet market demand is the intensification of the culture system. Intensified aquaculture systems can provide benefits both in terms of more products as well as the best use of resources. However, fish or shellfish under intensified culture could be exposed to stress, which, per se, negatively affects the immune system and causes diseases. Also, under circumstances like these, the cultured species may be under oxidative stress. It happens following the lack of balance between the production of ROS and antioxidant defense. Oxidative stress can cause DNA hydroxylation, protein denaturation, lipid peroxidation, apoptosis, and, ultimately, cell damage. Considering the fact that an increase in efficiency and levels of antioxidant defense has an influential role in mediating host benefits, there were extensive research attempts toward introducing novel and safe feed additives as a good alternative for synthetic antioxidants. In this sense, microbial feed additives can play a crucial role. Over the past years, there have been numerous studies on microbial feed additives, including probiotics, prebiotics and synbiotics. Most recently, to avoid the risks of the use of live microbes, the concept of paraprobiotics and postbiotics has been developed. These approaches focus on the use of dead, inactivated, or the products of probiotics consisting of a wide range of molecules, including peptidoglycans, surface proteins, cell wall polysaccharides, secreted proteins, bacteriocins, and organic acids. Such microbial feed additives not only can help the host to have a stronger antioxidant defense system but also can help the sustainability of the industry by enhancing immune response and disease resistance and decreasing the need for chemicals and antibiotics. On the other hand, thanks to the exogenous enzymes (e.g., cellulase, chitinase, etc.) produced by those microbes, the need for fish meal consumption can be decreased by increasing the levels of plant protein and staff in diet formulation or using novel protein sources such as insects. The present lecture trying to discuss the importance of microbial feed additives as novel approaches for present-day aquaculture. In addition, the proposed mechanism of action of microbial feed additives will be discussed. Also, the gap in the existing knowledge and the area that needs further research will be highlighted.

References

Hoseinifar, S.H.; Ashouri, G.; Marisaldi, L.; Candelma, M.; Basili, D.; Zimbelli, A.; Notarstefano, V.; Salvini, L.; Randazzo, B.; Zarantoniello, M. Reducing the use of antibiotics in European aquaculture with vaccines, functional feed additives and optimization of the gut microbiota. *Journal of Marine Science and Engineering* **2024**, *12*, 204.

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Hoseinifar, S.H.; Yousefi, S.; Van Doan, H.; Ashouri, G.; Gioacchini, G.; Maradonna, F.; Carnevali, O. Oxidative stress and antioxidant defense in fish: The implications of probiotic, prebiotic, and synbiotics. *Reviews in Fisheries Science & Aquaculture* **2020**, *29*, 198-217.

Zhang, H.; Ran, C.; Teame, T.; Ding, Q.; Hoseinifar, S.H.; Xie, M.; Zhang, Z.; Yang, Y.; Olsen, R.E.; Gatlin, D.M. Research progress on gut health of farmed teleost fish: a viewpoint concerning the intestinal mucosal barrier and the impact of its damage. *Reviews in Fish Biology and Fisheries* **2020**, 1-18.

Hoseinifar, S.H.; Sun, Y.; Wang, A.; Zhou, Z. Probiotics as means of diseases control in aquaculture, A Review of current knowledge and future perspectives. *Frontiers in Microbiology* **2018**, *9*, 2429.

Van Doan, H.; Hoseinifar, S.H.; Ringø, E.; Ángeles Esteban, M.; Dadar, M.; Dawood, M.A.O.; Faggio, C. Host-Associated Probiotics: A Key Factor in Sustainable Aquaculture. *Reviews in Fisheries Science & Aquaculture* **2019**, 1-27.

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OP22

Role of enterocin DD14 in prophylactic and therapeutic control of necrotic enteritis in broilers using *Enterococcus faecalis* 14 and its Δbac mutant

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Purpose

To demonstrate the efficacy of *Enterococcus faecalis* 14 in the control of induced necrotic enteritis in broilers.

Methods

Six groups of 504 broilers consisting of an infected untreated control (IUC) group, an infected and amoxicillin treated control (ITC) group, and groups receiving prophylactically (2 groups) or therapeutically (2 groups) *E. faecalis* 14 or its Δbac mutant were used.

Results

Administration of the coccidial inoculum allowed the lesions necessary for the occurrence of subclinical NE. After challenge with *Clostridium perfringens* 56, lesions were observed on D26 in all groups except ITC and those receiving prophylactically and therapeutically *E. faecalis* 14. On D27, only ITC and the group prophylactically treated with *E. faecalis* 14 were without lesions. Average body weight and daily weight gain remained lower in the treated groups compared to the ITC group, but there was a clear improvement in the period between D21 to D27, especially in the group prophylactically treated with *E. faecalis* 14. Metataxonomic analyses showed a positive effect of *E. faecalis* 14 in maintaining the diversity and richness of the intestinal microbiota, in contrast to ITC group and other conditions.

Conclusions

The prophylactically administration of *E. faecalis* 14 reduced NE lesions caused by *C. perfringens*.



Abstracts

Selected Contributions

for

Oral Presentation



SP23

Contextual effect and safety of probiotics consumption in intestinal inflammatory processes

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The intestinal mucosa is constantly exposed to various environmental antigens, originating from pathogens, or of harmless origin such as dietary components or antigens from the commensal microbiota, which affect immune homeostasis. Therefore, the immune system associated with the intestinal mucosa requires a complex cellular network of highly regulated mechanisms in order to maintain tissue balance and, at the same time, protect against the entry of pathogens. Therefore, the immune system associated with the intestinal mucosa requires a complex cellular network of highly regulated mechanisms in order to maintain tissue balance and, at the same time, protect against the entry of pathogens. Such mechanisms include specialized dendritic cells (DCs) (which express the CD103+CD11b- and CD103+CD11b+ molecules) that induce canonical responses, such as regulatory T cells (Treg) that control inflammatory responses and promote tolerance to harmless antigens, and effector cells (particularly Th17 lymphocytes) that promote tissue-specific barrier immunity. Such barrier response encompasses specialized epithelia, the production of antimicrobial molecules, antibodies, and mononuclear phagocytes. Although the intestinal mucosal immune system is constantly shaped by host genetic factors, resident microbiota, dietary habits, and exposure to environmental pathogens, the result of these interactions is usually a return to tissue homeostasis. However, disruption of the balance between tolerance and barrier immunity can lead to chronic inflammatory diseases, such as inflammatory bowel diseases (IBD). In the Mucosal Immunology Lab, we have been studying how probiotics, dietary changes, or infection episodes, shape the gut-associated mucosal immune system. We observed that, after infection clearance, *Yersinia pseudotuberculosis* (YP) causes a permanent remodeling of the immune and lymphatic systems of the gastrointestinal tract. This process, named by us “immunological scarring”, is directly related to susceptibility to experimental colitis as it compromises the migration of tolerogenic dendritic cells (DCs) to mesenteric lymph nodes. Here, we tried to reverse the immunological scarring by using two different supplements: (1) an inactivated *Saccharomyces cerevisiae* strain enriched with selenium (Se) and (2) the active yeast *S. cerevisiae* (SC) (strain UFMG A-905). Our group hypothesized that both treatments could recover the mesenteric lymphatic integrity and the migratory capacity of the CD103+ DCs, crucial for inducing tolerogenic responses in the intestine. Testing on C57BL/6 mice involved treatment for 2 weeks post-infection, to reverse the immunological scar, continuing until pathogen elimination to prevent its establishment. The results obtained until now showed that post-infection treatment with the yeast SC UFMG A-905, contrary to our initial hypothesis, aggravated chronic inflammation in previously infected animals, indicated by increased

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recruitment of neutrophils and Th1 cells to the mesentery and mesenteric lymph nodes (MLNs) post-infection and animal mortality. We also observed that neither treatment was sufficient to restore the integrity of the mesenteric lymphatic vessels, since the treated animals also presented reduced frequency of CD103+CD11b⁻ and CD103+CD11b⁺ DCs in the MLNs. Although these treatments have proven efficacy against intestinal inflammatory processes, we hypothesize that their beneficial effect on the mucosa is contextual and that probiotics consumption during immunological scarring may further weaken the intestinal barrier.

Keywords: Mucosal Immunology; Probiotics; Experimental colitis; Inflammation

References:

- FONSECA, D. DA et al. Microbiota-Dependent Sequelae of Acute Infection Compromise Tissue-Specific Immunity. *Cell*, v. 163, n. 2, p. 354–366, out. 2015.
- SAMANTHA et al. Evaluation of a functional craft wheat beer fermented with *saccharomyces cerevisiae* UFMG a-905 to treat salmonella typhimurium infection in mice. *Probiotics and antimicrobial proteins*, v. 15, p. 1180—1192, 2023.
- DE CAMPOS FRAGA-SILVA, T. F. et al. Selenization of *S. cerevisiae* increases its protective potential in experimental autoimmune encephalomyelitis by triggering an intestinal immunomodulatory loop. *Scientific Reports*, v. 10, p. 22190, 17 dez. 2020.
- AM;LAMMERS, S. Multiple Lactobacillus Infections Caused by Probiotics at Pediatric and Adult Academic Medical Centers. *WMJ : official publication of the State Medical Society of Wisconsin*, v. 123, n. 4, set. 2024.

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SP24 Evaluation for optimal conditions of γ -aminobutyric acid production by *Lactiplantibacillus plantarum* ST0414

Evaluation for optimal conditions of γ -aminobutyric acid production by *Lactiplantibacillus plantarum* ST0414

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Lactic acid bacteria are known for their beneficial properties, including production of various antimicrobials and health promoting metabolites, such as γ -aminobutyric acid (GABA), a non-protein amino acid that acts as a neurotransmitter, produced mainly through the decarboxylation of glutamate by the enzyme glutamate decarboxylase. *Lactiplantibacillus plantarum* is a species applied in the fermentation of dairy, meat and plant-based products, where plays an essential role as starter, adjunct, protective or probiotic/postbiotic culture, increasing functional properties in the synthesis of its metabolites, such as GABA.

The objective of this study was to evaluate safety and GABA production by *Lpb. plantarum* ST0414 and explore influence of the culturing conditions such as cell density of the producer, concentration of monosodium glutamate (an GABA precursor), pH, temperature and incubation time. Expressed GABA was quantified by gas chromatography coupled to mass spectrometry (GC-MS).

In this study we have studied *Lpb. plantarum* ST0414 strains, isolated from Bulgarian white brine cheese produced by medium scale producers and identified based on its biochemical and genetic characteristics including 16S rRNA sequencing. The strain ST0414 was selected for current study based on preliminary screening for GABA production between more than 150 bacterial isolates, most of them obtained from dairy products. GABA production from *Lpb. plantarum* ST0414 was evaluated in different levels of cellular density (10^5 - 10^8 CFU/ml), monosodium glutamate concentration (30 mM-120 mM), pH (4.0-8.0), temperatures (from 30°C to 50°C) and incubation time (24h-120h), varying one method at a time, maintaining other standard conditions of 10^6 UFC/ml, 60 mM, pH 6.0, 37°C and 24h and following to additional experimental set-ups. Cell free supernatant was obtained after centrifuged at $15.000\times g$ for 15 minutes at 20°C, diluted $10\times$, derivatized in triplicate with methyl chloroformate and quantified by GC-MS.

Comparing results obtained from the different experiments following preselected variables, the production of GABA by *Lpb. plantarum* ST0414 was present in superior levels when was subjected to a longer incubation time 120h (27.98 mg/ml) and microbial density of 10^5 CFU/ml



(16.13 mg/ml), respectively. Extreme temperatures of 30°C and 50°C used demonstrated better results (13.64 mg/dl and 14.27 mg/dl, respectively), being assimilated to the greater production of GABA under stress conditions by the ST0414 strain. Variations in pH and glutamate concentration did not prove to be influencing factors in the production of GABA, maintaining close values between their respective variations. Moreover, based on performed biochemical and physiological tests, hemolytic, gelatinase, proteolytic and lipolytic activity, biogenic amines production and antibiotic resistance, including different *van* genes, *Lpb. plantarum* ST0414 can be considered as a safe strain.

Obtained results, suggest that GABA production can be increased when *Lpb. plantarum* ST0414 is exposed to stress growth conditions. In our current study we have recorded that *Lpb. plantarum* ST0414 has optimal conditions for GABA production with 120h of incubation, cell density of 10^5 UFC/ml and temperature of 30°C or 50°C. Thus, information can be further applied and scaled with aim of biotechnological production where *Lpb. plantarum* ST0414 can be explored as an industrial GABA producer.

References:

RAWLINSON, C. et al. A rapid method for profiling of volatile and semi-volatile phytohormones using methyl chloroformate derivatization and GC–MS. *Metabolomics*, v. 11, n. 6, p. 1922–1933, 1 dez. 2015.

ZHANG, Q. et al. Characterization of γ -aminobutyric acid (GABA)-producing *Saccharomyces cerevisiae* and coculture with *Lactobacillus plantarum* for mulberry beverage brewing. *Journal of Bioscience and Bioengineering*, v. 129, n. 4, p. 447–453, 1 abr. 2020.

ZHUANG, K. et al. Transcriptomic response to GABA-producing *Lactobacillus plantarum* CGMCC 1.2437T induced by L-MSG. *PLOS ONE*, v. 13, n. 6, 1 jun. 2018.

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SP25

Effects of synbiotics, protein supplementation associated to resistance training on inflammation, oxidative stress, and muscle strength in older adults with type 2 diabetes mellitus: A triple-blinded randomized

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Background & aims: The accelerated ageing of the population represents a significant achievement of contemporary society; however, it also poses a series of challenges, particularly for developing countries, including the rise in non-communicable diseases, which can significantly increase the burden on healthcare and care services. Brazil ranks sixth globally in the number of individuals with type 2 diabetes mellitus (T2DM), with projections estimating 23.2 million cases by 2045. This places Brazil among the top ten countries with the highest prevalence of T2DM. There is an increased risk of sarcopenia with aging and T2DM, highlighting the involvement of diabetes in the pathophysiology of the musculoskeletal diseases. In addition to a low protein intake and sedentary habits, recent findings indicate that microbial dysbiosis contributes to systemic oxidative stress and the inflammaging pattern, already observed in aging process. Changes in the lifestyle of individuals with T2DM are crucial along with pharmacological interventions to improve the overall health status, since these modifications may also regulate the redox and inflammatory scenario.

Objective: This study investigated the combined effects of Protein supplementation, Synbiotic+Protein and Resistance Training (RT) on physical performance, body composition, glycemic, inflammatory and oxidative stress parameters in older adults with T2DM.

Methods: Randomized, triple-blinded clinical trial involving men aged over 65 with T2DM submitted to 12-week muscle-strength exercise protocol involving twice-weekly supervised sessions lasting 45-60 minutes. Participants (n=51) were allocated in three experimental groups: Control (only RT), Protein (20g of whey protein after RT) and Synbiotic+Protein (commercial capsules containing 10×10^{10} UFC/ml of *Lactobacillus acidophilus* NCFM, *Lactocaseibacillus paracasei* Lpc-37, *Bifidobacterium lactis* BI-07, *Bifidobacterium lactis* BI-04 and microcrystalline cellulose plus 20g of whey protein).

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Results: Participants showed homogeneity in bio-sociodemographic characteristics, although the SP group had lower alcohol consumption and Protein group reported higher use of sulfonylurea medication, potentially explaining the higher fat mass, body mass index and body fat percentage. All groups showed improved physical performance, and better redox balance after interventions, as indicated by higher uric acid levels (an antioxidant) and lower pro-oxidant iron concentrations in plasma. Although all groups showed higher levels of muscle injury markers (lactate dehydrogenase and creatine kinase activities), only the Protein group demonstrated strength gains, reduced insulin resistance, and oxidative insults from heme-iron species. Pro-inflammatory cytokines IFN- γ and IL-6 were also increased in the Protein group, possibly reflecting their role as mediators of integrative energy metabolism responses. The Synbiotic+Protein group exhibited reduced IFN- γ and increased Heme-Iron and CK concentrations.

Conclusions: Resistance Training combined with Protein supplementation promoted strength gain and improved physical performance improvement some markers for inflammation and oxidative stress while the type and dosage used of Synbiotic combined with Protein and Resistance Training did not bring additional advantages.

Keywords: Aging; Sarcopenia; Inflammation mediators; Exercise

References:

- Miller EG, Nowson CA, Dunstan DW, Kerr DA, Menzies D, Daly RM. Effects of whey protein plus vitamin D supplementation combined with progressive resistance training on glycaemic control, body composition, muscle function and cardiometabolic risk factors in
Tamayo M, Olivares M, Ruas-Madiedo P, Margolles A, Espín JC, Medina I, Moreno-Arribas MV, Canals S, Mirasso CR, Ortín S, Beltrán-Sanchez H, Palloni A, Tomás-Barberán FA, Sanz Y. How Diet and Lifestyle Can Fine-Tune Gut M
Qaisar R, Burki A, Karim A, Iqbal MS, Ahmad F. Probiotics Supplements Improve the Sarcopenia-Related Quality of Life in Older Adults with Age-Related Muscle Decline. *Calcif Tissue Int.* 2024 Jun;114(6):583-591. doi: 10.1007/s00223-024-01211-6. Epub 2024 Ap
Wu J, Yang K, Fan H, Wei M, Xiong Q. Targeting the gut microbiota and its metabolites for type 2 diabetes mellitus. *Front Endocrinol (Lausanne)* 2023;14. <https://doi.org/10.3389/fendo.2023.1114424>.
Meuffels FM, Isenmann E, Strube M, Lesch A, Oberste M, Brinkmann C. Exercise Interventions Combined With Dietary Supplements in Type 2 Diabetes Mellitus Patients—A Systematic Review of Relevant Health Outcomes. *Front Nutr* 2022;9. <https://doi.org/10.>

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SP26

Local artisanal dairy fermented products as reservoir of beneficial microorganisms

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Beneficial microorganisms provide added value to food products or hosts and correspond to what is often referred to as "positive flora". These microorganisms exhibit diverse biological activities and produce metabolites of interest, which can positively impact food preservation, flavor development, and host health. Beneficial microorganisms can generally be classified into two main categories: antagonistic microorganisms and probiotic microorganisms.

Antagonistic microorganisms are those that inhibit undesirable microorganisms, by either active or passive competition. Active antagonism involves the production of bioactive molecules, such as bacteriocins, organic acids, or antifungal compounds, which directly suppress harmful organisms. Passive competition, on the other hand, relates to microorganisms occupying ecological niches or competing for nutrients without necessarily producing active molecules. These mechanisms have been extensively studied in bacteria; however, research on yeasts exhibiting similar antagonistic properties remains relatively limited and is a growing area of interest.

Probiotic microorganisms, in contrast, must demonstrate a beneficial biological activity *in vivo* within a host. These microorganisms, including certain bacterial and yeast strains, are capable of promoting gut health, improving immunity, and supporting overall host well-being. Fermented foods, such as artisanal cheeses, are excellent examples of how beneficial microorganisms can enhance food preservation while simultaneously providing health and sensory benefits. During the ripening process of traditional cheeses, complex microbial ecosystems develop, consisting of bacteria, yeasts and molds. These organisms play a crucial role in flavor development, texture formation and the inhibition of spoilage flora or pathogens. For example, in the Hauts-de-France region of the north of France, the artisanal cheese Tomme d'Orchies was studied using metagenetic analysis during its ripening process. This analysis revealed the microbial diversity present in both the rind and core of the cheese and highlighted the evolution of bacterial and yeast microbiota over time. Notably, two strains of *Kluyveromyces* isolated from this cheese demonstrated interesting antagonistic activities.

Further studies on other regional cheeses, such as Carré du Vinage and Bourle Roncquoise, identified and isolated antimicrobial strains of yeasts and bacteria. Two specific yeast strains were tested in a curd model, where they demonstrated significant biopreservation potential by inhibiting spoilage microorganisms. These results suggest that such yeasts could be valuable for extending the shelf life and safety of cheeses.

Fermented foods represent a significant reservoir of beneficial microorganisms with wide-ranging applications. Beyond food biopreservation, these microorganisms have potential uses in improving animal health, human gut health, and even reducing food waste by preventing



spoilage. Artisanal cheeses, therefore, serve as both cultural heritage and a valuable platform for exploring microbial diversity and its positive impacts on food systems and health.

Keywords: biopreservation, antagonism, probiotics, fermented food

References

Commenges A, Lessard MH, Coucheney F, Labrie S, Drider D. 2024. The biopreservative properties of *Metschnikowia pulcherrima* LMA-2038 and *Trichosporon asahii* LMA-810 in a model fresh cheese, are presented. *Food Bioscience*.58: 103458. doi : 10.1016/j.fbio.2

Ceugniez A, Coucheney F, Jacques P, Daube G, Delcenserie V, Drider D. 2017. Anti-Salmonella activity and probiotic trends of *Kluyveromyces marxianus* S-2-05 and *Kluyveromyces lactis* S-3-05 isolated from a French cheese named “Tomme d’Orchie”



SP27 Functional Minas frescal cheese with spore-forming *Weizmannia coagulans* GBI-30

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The effect of the addition of *Weizmannia coagulans* BC30 on the probiotic survival and tolerance in the gastrointestinal tract, the anti-pathogenic activity along the storage time (1, 7, 14 days, spot diffusion antimicrobial susceptibility test) and the generation of bioactive compounds (antioxidant, antihypertensive and antidiabetic values) considering three different dosages (6-7, 8-9, 10-11 log CFU/g, respectively, QII, QIII, QIV) was studied. For comparison, a conventional cheese was also produced without the addition of *W. coagulans* BC30 (QI). Pathogenic strains of *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19,117 and *Salmonella enterica* subsp. *diarizonae* ATCC 12,325, all from clinical or dairy isolates, were used to evaluate the antibacterial activity of the probiotic cheeses. The non-pathogenic strain *Listeria innocua*, a surrogate for *Listeria monocytogenes*, was also used. *W. coagulans* showed good survival (>6 log UFC/g) and tolerance to gastrointestinal disorders throughout the storage period. The probiotic count after passing through the GIT conditions fluctuated between 6.12 and 6.71, 7.34–7.89, and 8.12–8.56 log CFU g⁻¹ for QI, QII, and QIII, respectively, proving to be constant and proportional to the concentration of probiotics added to the samples. This observation confirms that Minas Frescal cheese is an excellent food matrix for maintaining probiotic viability and protecting against adverse conditions in the gastrointestinal tract. The antioxidant activity (DPPH), antihypertensive activity (angiotensin-converting enzyme inhibitory activity, ACEi), antidiabetic activity (α -amylase and α -glucosidase, and proteolytic activity showed a similar behavior over the storage time of the probiotic cheeses, with higher inoculation concentrations generating a correspondingly higher bioactive and proteolytic activity ($p < 0.05$). During refrigerated storage, the concentration of bioactive peptides and proteolytic activity increased in proportion to the concentration of probiotic bacteria in the food matrix (QIII > QII > QI), while to the conventional cheese (QC), the concentration remained lower values and stable over the 14 days ($p > 0.05$). Inhibition zones < 2 mm were associated with low antagonistic activity, 2-5 mm with medium antagonistic activity, and > 5 mm with high antagonistic activity. Low antimicrobial activity was observed against *S. aureus* (0.6-1.8 mm) and *L. innocua* (0.6-1.8 mm) strains, while moderate activity was observed against *Salmonella* (0.6-2.4 mm) and *E. coli* (0.6-3.0 mm). The antibacterial activity was enhanced as the concentration of *W. coagulans* increased along the storage time in both antagonism tests. The antibacterial activity of *W. coagulans* may be related to the production of lactic acid, bacteriocin and hydrogen



peroxide. These results suggest that it is possible to add a spore-forming probiotic bacterium to a fresh cheese, with adequate survival along the gastrointestinal tract and viability in the final product throughout the storage period, capable of producing functional and antibacterial compounds

Keywords: fresh cheese, functional dairy food, spore-forming bacteria

References:

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SP28 **Gut microbiome composition and its association with dietary factors in Crohn's disease**

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Introduction: The gut microbiota plays a crucial role in the pathogenesis and progression of Crohn's Disease (CD) by maintaining intestinal homeostasis, supporting metabolic and immune functions, and influencing overall health. An imbalance in the gut microbiota is associated with CD, as disruptions in microbial composition can be further impacted by dietary factors, highlighting the connection between nutrition and gut health.

Aim: This study aimed to evaluate the gut microbiota composition and its interactions with dietary factors to identify potential opportunities for targeted probiotic interventions in CD management.

Methods: The study included 53 participants, of whom 20 had CD in remission, 18 had active disease, and 15 were healthy controls. CD activity was evaluated by colonoscopy assessment or by nuclear magnetic resonance scan enterography. All participants aged between 18 and 60 years were assessed using 24-hour dietary recall and gut microbiome profiling through 16S rRNA gene sequencing. Patients had confirmed diagnoses of CD with ileal and/or colonic involvement. The local ethics committee approved this study.

Results: Microbial composition analyses (beta diversity) revealed significant differences between groups. At the Operational Taxonomic Unit (OTU) level, healthy microbiome differed significantly from both remission and active CD patients, with healthy controls showing a more similar microbial composition compared to remission ($p = 0.001$) and active disease ($p = 0.001$). In addition, Shannon diversity analyses demonstrated that healthy individuals exhibited significantly higher OTU diversity compared to CD patients, further highlighting the microbial imbalance associated with the disease. Among specific taxa, *Eubacterium hallii*, a key butyrate producer, was significantly reduced in patients with active CD. This microorganism is integral to intestinal metabolic balance, playing essential roles in energy homeostasis, colonic motility, immunomodulation, and inflammation suppression. Furthermore, *E. hallii* plays a critical role in bile acid and cholesterol metabolism, highlighting its importance in gut health. Disease severity was associated with changes in microbial abundance, with specific taxa colonizing the gut at higher levels as disease activity increased, including *Escherichia coli*, *Escherichia-Shigella*, and taxa from the *Enterobacteriaceae*, *Lachnospirales*, and *Bacteroides*. This growth may be linked to inflammatory processes and intestinal dysbiosis, suggesting a potentially pathogenic role. Furthermore, dietary components such as saccharin, an artificial sweetener, were significantly associated with alterations in microbial composition.

Conclusions: These findings underline the potential for probiotics targeting specific taxa, such as *E. hallii*, to restore microbial balance and improve clinical outcomes in CD. The study also highlights the impact of dietary components, such as saccharin, in disrupting the gut



microbiome, suggesting that certain dietary factors may contribute to microbial imbalance and inflammation in CD. Future research should focus on integrating dietary patterns with probiotic development to optimize gut health and mitigate inflammation in CD patients.

Keywords: Crohn's disease; gut microbiome; nutrition; probiotics

References:

BOLTE, L. A. et al. Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. *Gut*, p. gutjnl-2020-322670, 2 abr. 2021.

FAN, Y.; PEDERSEN, O. Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, v. 19, n. 1, p. 55–71, 4 jan. 2021.

GILL, P. A. et al. The Role of Diet and Gut Microbiota in Regulating Gastrointestinal and Inflammatory Disease. *Frontiers in Immunology*, v. 13, n. April, p. 1–22, 5 abr. 2022.



Abstracts

Posters



PP030: Effects of a 217 km ultramarathon on the composition of the gut microbiota of a runner with a low-fiber diet

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Moderate physical activity can positively change the gut microbiota (GM). It is known that ultramarathon, an extreme physical exercise, causes disturbances of physiological homeostasis and GM in runners. Diet is the most important modulator of GM. A high-fiber diet is associated with the proliferation of bacteria that produce short-chain fatty acids, which could be beneficial for human health. The aim of this study was to investigate the change in GM composition of a runner on a low-fiber diet after a world ultramarathon. The study was conducted during the 2021 edition of the Brazil 135 Ultramarathon (BR135), a single-stage mountain ultramarathon over a distance of 217 km. A food frequency questionnaire and a stool sample were collected 7 days before the race (T0). Two further stool samples were collected 15 minutes after the race (T1) and 7 days after the race (T2). The GM was analyzed by metagenomic shotgun sequencing to assess the microbial composition across the different time points. A male ultramarathon runner with a BMI of 27.87 kg/m², 44 years old, on a low-fiber diet (7 g/day) and ranked 101st in BR135, was studied. α -diversity remained stable after the race, followed by a decrease at T2. The Bacillota/Bacteroidota ratio increased after the race. No Bacteroidota detected 7 days after the race. The proliferation of Pseudomonadota and Verrucomycetota was observed at T1. The presence of Veillonellaceae was not detected. At the genus level, *Blautia* did not change post-race, but showed a clear increase in T2. At the species level, higher proportions of *Eubacterium rectale* and *Ruminococcus bromii* were observed at the beginning, which decreased slightly after the race, followed by a notable decrease at T2. In contrast, *Blautia wexlerae* and *Fusicantennibacter saccharivorans* remained stable after the race, followed by a strong increase 7 days after BR135. The decrease in α -diversity combined with the increased Bacillota/Bacteroidota ratio could indicate a disruption of GM homeostasis. The increase in the proportion of Pseudomonadota could be related to a high protein intake, which may explain the decrease in fiber-fermenting bacteria. Taxa of this phylum can synthesize metabolites harmful to human health, such as TMAO. The absence of Veillonellaceae contrasts with previous findings of increased numbers of *Veillonella* species in ultramarathon runner. They are thought to have an ergogenic effect on endurance by converting lactate into short-chain fatty acids. In addition, a reduction in *Eubacterium rectale* and *Ruminococcus bromii*, an important fiber-fermenting species, possibly due to low fiber intake, which may affect gut health by reducing butyrate production. The marked increase in *Blautia* spp. at T2, suggesting a delayed response of this species to the physiological stress of the ultramarathon or to nutritional factors during



the recovery phase. In conclusion, the combination of extreme physical exertion during BR135 and a low-fiber diet may have negatively impacted the runner's GM, suggesting a possible detrimental effect on gut health after an ultramarathon.

References

- COUTINHO-WOLINO, Karen Salve et al. Can diet modulate trimethylamine N-oxide (TMAO) production? What do we know so far?. *European journal of nutrition*, p. 1-18, 2021.
- NILSEN, Morten et al. Butyrate levels in the transition from an infant-to an adult-like gut microbiota correlate with bacterial networks associated with *Eubacterium rectale* and *Ruminococcus gnavus*. *Genes*, v. 11, n. 11, p. 1245, 2020.
- SHIN, Na-Ri; WHON, Tae Woong; BAE, Jin-Woo. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends in biotechnology*, v. 33, n. 9, p. 496-503, 2015.
- GROSICKI, Gregory J.; DURK, Ryan P.; BAGLEY, James R. Rapid gut microbiome changes in a world-class ultramarathon runner. *Physiological reports*, v. 7, n. 24, p. e14313, 2019.

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PP031: Potential Probiotic Bacteria from Kefir Grains: Isolation, Identification by MALDI-TOF MS and In Vitro Gastrointestinal Resistance

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The isolation of new microorganisms is essential for biotechnological advances in the pharmaceutical, food, cosmetic and environmental sectors. Kefir, a beverage fermented by bacteria and yeasts, presents a promising microbial diversity for obtaining potential probiotic microorganisms adapted to various substrates. Evaluating gastrointestinal resistance in vitro is fundamental for selecting microorganisms that are beneficial to health, as they need to survive the acidic pH of the stomach, bile salts and intestinal enzymes in order to colonize the host, at least temporarily. The aim of this study was to isolate, identify and evaluate the gastrointestinal resistance of lactic acid bacteria from kefir grain microbiota. Homemade kefir grains, from Uberaba/MG and Araraquara/SP, were cultured daily in UHT milk (1:100 m/v) and incubated at 30°C until reaching pH 4.5 ± 0.2 . For lactic acid bacteria (LAB) isolation, 1 g of each grain was macerated and diluted in 0.1% (m/v) peptone water, followed by serial dilutions. The samples were plated on MRS and M17 agar, then incubated at 37°C for 48 h under anaerobic conditions. Morphologically distinct colonies were isolated and subcultured to obtain pure cultures, followed by Gram staining and catalase testing for preliminary characterization. Bacterial identification was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). *In vitro* gastrointestinal resistance was evaluated using the static INFOGEST protocol, with assessment of bacterial viability after the initial, oral, gastric and intestinal phases. The data were evaluated by one-way ANOVA and Tukey's post-test ($p < 0.05$). A total of 109 isolates were selected from the kefir microbiota, including 35 Gram-positive and catalase-negative bacteria. In Araraquara/SP grains, *Enterococcus durans* (6), *Enterococcus faecium* (4), and *Lentilactobacillus kefir* (1) were identified. Belo Horizonte/MG grains contained *Enterococcus faecium* (22), *Lentilactobacillus kefir* (1), and *Lactococcus lactis* (1). These microorganisms are commonly associated with food products. However, the *Enterococcus* spp. genus is controversial due to its potential production of virulence factors and antibiotic resistance. Only bacteria belonging to different genera were selected for evaluation of gastrointestinal resistance. The *L. lactis* strain had an initial population of 7.71 ± 0.16 log CFU/mL, but did not survive the gastric and intestinal phases. The other strains showed reductions of approximately 1 log compared to the initial population ($p < 0.05$), with different sensibility to the oral, gastric and intestinal phases. *E. durans* decreased from 7.80 ± 0.16 to 6.87 ± 0.14 ; *E. faecium* from 7.59 ± 0.75 to 6.64 ± 0.47 ; *L. kefir* from 7.53 ± 0.03 to 6.53 ± 0.08 log CFU/mL. The results show that the three strains selected from kefir grains are resistant to the simulated gastrointestinal environment, an important condition for providing systemic beneficial effects. The selected strains will be tested for antimicrobial activity, antibiotic susceptibility and cholesterol-lowering capacity in vitro, before being used for technological purposes.



References

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO); WORLD HEALTH ORGANIZATION (WHO). Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. 2002. Disponível em: <https://www.fao.org/3/a051>

HURTADO-ROMERO, Alejandra et al. Probiotic Properties, Prebiotic Fermentability, and GABA-Producing Capacity of Microorganisms Isolated from Mexican Milk Kefir Grains: a clustering evaluation for functional dairy food applications. *Foods*, [S.L.], v. 10, n

MANTEGAZZA, Giacomo et al. Use of kefir-derived lactic acid bacteria for the preparation of a fermented soy drink with increased estrogenic activity. *Food Research International*, [S.L.], v. 164, p. 322-345, fev. 2023. Elsevier BV. <http://dx.doi.org/10.101>

MINEKUS, M. et al. A standardised static in vitro digestion method suitable for food – an international consensus. *Food Funct.*, [S.L.], v. 5, n. 6, p. 1113-1124, 2014. Royal Society of Chemistry (RSC). <http://dx.doi.org/10.1039/c3fo60702j>

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PP032: Probiotic viability by flow cytometry and plate counting: a comparative analysis of the methods.

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The intestinal microbiota plays a fundamental role in human health, influencing various physiological processes. Imbalances in this microbiota, known as dysbiosis, are associated with several diseases. Probiotics, by rebalancing the intestinal microbiota, can contribute to the prevention and treatment of various health conditions. The consumption of dietary supplements, especially probiotics, saw significant growth during the COVID-19 pandemic. The strengthening of the immune system drove the demand for these beneficial microorganisms. However, maintaining the viability of probiotics during production, storage, and passage through the gastrointestinal tract presents challenges for the industry. Traditionally, plate counting has been the standard method for quantifying viable microorganisms. Despite its widespread use, this technique has limitations, such as prolonged incubation times and underestimation of the total number of viable cells. Flow cytometry has emerged as a faster and more accurate alternative. This study aims to compare the cell viability of a probiotic strain using two counting techniques: traditional plate counting and flow cytometry. Samples from three batches of tablets were subjected to cell counting comparisons using both techniques before the simulated *in vitro* digestion process. The probiotic strain *Lactocaseibacillus rhamnosus* GG (ATCC 53103) was used at a concentration of 10 billion cells. In addition to the counts performed, the morphology of the cells was observed through optical microscopy. The values obtained from the plate counting technique were batch A1: 9.12; batch A2: 9.39; batch A3: 8.77 log cfu/g. In comparison, the results from the active fluorescent cell units (afu) were batch A1: 10.61; batch A2: 10.58; batch A3: log 10.61 afu/mL. It is observed that the results obtained by flow cytometry showed higher counts of the probiotic. This observed difference can be explained by the condition that, in the plate counting technique, colonies can be formed from both individual cells and clustered cells, which may lead to an underestimation of the actual value, whereas in flow cytometry, each cell passing through the set of lasers in the equipment is counted, leading to a more accurate value. Flow cytometry has proven to be more sensitive for analyzing the cell viability of probiotics than standard plate counting, as it allows for the analysis of thousands of cells per second, providing real-time results where multiple cellular characteristics can be evaluated simultaneously using specific markers. Additionally, flow cytometry provides quantitative data about cellular populations, making it essential for improving research on new strains. In the morphological analysis, Gram-positive rods and the formation of some cellular clusters were observed, confirming our hypothesis that the counting of cfus may have been underestimated compared to the counting in afus.



References

ISO 19344: IDF 232 - Milk and milk products–Starter cultures, probiotics and fermented products–Quantification of lactic acid bacteria by flow cytometry. International Standards Organisation (ISO) & International Dairy Federation (IDF); 20

USP, United States Pharmacopeia (2024). Dietary Supplement Monographs, Lacticaseibacillus rhamnosus. USP-NF. Rockville, MD: United States Pharmacopeia. DOI: https://doi.org/10.31003/USPNF_M10467_04_01

CARVALHO A.T., FERRAZ R., RIBEIRO G.A. e BERTHO A.L. - Citometria de Fluxo no estudo das doenças infecto-parasitárias. Curso de Inverno de Citometria de Fluxo do Instituto Oswaldo Cruz, FIOCRUZ, 2010. Disponível em: < Apostila de Citometr

FAO/WHO. Probiotics in food: health and nutritional properties and guidelines for evaluation: report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food, including powder milk with live lactic ac

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PP033: FUNCTIONAL OAT BEVERAGE WITH EMPHASIS ON THE CONTROL OF CARIES AND PERIODONTITIS MICROORGANISMS

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Caries and periodontitis are diseases associated with dysbiosis that can compromise children's oral health, causing pain, bleeding gums, impaired feeding, and weight/growth gain due to inadequate nutrient intake. Some studies have shown microorganisms with an inhibitory effect on *Streptococcus mutans* – the main cause of caries – and *Porphyromonas gingivalis* – a pathogen associated with periodontitis. Among them, *Lactobacillus rhamnosus* LRB and *Lactobacillus rhamnosus* SP1, are species with potential inhibitory action in both species. Thus, the present project aims to develop a functional oat beverage and evaluate potential inhibitory effects on species associated with oral diseases. The beverage developed is based on oats, the prebiotic inulin and xylitol, a non-cariogenic sweetening agent. For the development of the beverage, viability tests were carried out, which demonstrated the permanence of the amount of bacteria *Lb. rhamnosus* LRB in $\log 8,01 \pm 0,78$ and *Lb. rhamnosus* SP1 in $\log 8,25 \pm 0,37$, for 28 days in refrigeration ($\leq 4^{\circ}\text{C}$); pH was kept slightly below 4.5 and the titratable acidity curve at 0.5% on the day of fermentation and remained between 0.6 and 0.77% throughout the fermentation and storage period. In addition, through the digestion protocol of the beverage 16 days of conservation, it was observed that, in the digestion process, *Lb. rhamnosus* SP1 log had a small variation between the salivary (7,57 log), gastric (7,17 log) and intestinal phases (6,92 log) and remained very close to the viability value before digestion (7,94 log). The *Lb. rhamnosus* LRB, in the salivary phase (log 8,13), remained very close to the viability value before digestion (log 7,97), but in the gastric and enteric phases it did not obtain a result as high as that of *Lb. rhamnosus* SP1. In vitro analyses were also performed, verifying the production of bacteriocins by the strains and concluding that there was no action against *S. mutans* and *P. gingivalis*. In addition, it was observed that, through antibiogram analysis, in the plaques inoculated with *Lb. rhamnosus* SP1, a halo of inhibition was observed in the antibiotics Kanamycin, Tetracycline, Tylosin, Clindamycin, Erythromycin, Chloramphenicol, Ampicillin, Gentamicin and Streptomycin and resistance against Vancomycin and Nalidixic acid. In the case of *Lb. rhamnosus* LRB, halo of inhibition was generated in the antibiotics Kanamycin, Tetracycline, Chloramphenicol, Ampicillin and Gentamicin and resistance to Streptomycin, Vancomycin, Tylosin, Nalidixic Acid, Clindamycin and Erythromycin. Therefore, the proposed functional oat beverage is promising based on the results observed so far, but further analysis is still needed to verify its functionality for the microbial ecology in the mouth, which is related to the composition of the bacteria present, preserving the bacteria associated with health and reducing the bacteria associated with periodontitis and caries. These results require



as next steps the verification of biofilm formation and competition between microorganisms, as well as the mimicking of the oral cavity of children in The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) to simulate the passage of the beverage in this environment and biofilm evaluation before and after treatment.

References

ABDELRAHMAN, M.; HSU, K. L.; MELO, M. A.; DHAR, V.; TINANOFF, N. Mapping evidence on early childhood caries prevalence: complexity of worldwide data reporting. *International journal of clinical pediatric dentistry*, v. 14, n.1, 2021.

BISWAS, S.; TURNER, L.; BISWAS, I. Lactobacillus rhamnosus LRB mediated inhibition of oral streptococci. *Molecular Oral Microbiology*, [S. l.], v. 33, n. 5, p. 396–405, 2018. a. DOI: 10.1111/omi.12242.

RODRÍGUEZ G, RUIZ B, FALEIROS S, et al. Probiotic Compared with Standard Milk for High-caries Children: A Cluster Randomized Trial. *Journal of Dental Research*. 2016;95(4):402-407. doi:10.1177/0022034515623935

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PP034: KEGGaNOG: A Streamlined Solution for KEGG Pathway Annotation

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The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a foundational resource for annotating and understanding the metabolic pathways of microbes and microbial communities, particularly in gut microbiome research. Despite its value, leveraging KEGG pathway annotations often involves time-intensive workflows or reliance on commercial tools like DRAM [1], which can limit accessibility for many researchers. Tools such as eggNOG-mapper offer rapid and comprehensive functional annotation, which includes the annotation of KEGG pathways of novel sequences but lack built-in visualization capabilities [2]. Conversely, KEGG-Decoder excels in visualizing KEGG pathways but requires outputs from KEGG-Koala – a web-based tool with extended processing times and limited flexibility [3]. These limitations can create bottlenecks for researchers, particularly those working on large-scale studies. To bridge this gap, we introduce KEGGaNOG, an open-source tool that integrates eggNOG-mapper annotations with KEGG-Decoder for a seamless annotation and visualization workflow.

KEGGaNOG is designed to parse eggNOG-mapper outputs and format them to be compatible with KEGG-Decoder, enabling researchers to generate heatmaps of KEGG pathway presence and completeness efficiently. This integration transforms the annotation and visualization process into a single, streamlined workflow that minimizes the need for manual intervention or complex post-processing. Furthermore, when paired with a Snakemake pipeline [4], KEGGaNOG and eggNOG-mapper enable one-click metabolic profiling, significantly reducing the complexity and time required for analyzing gut microbiome datasets.

KEGGaNOG is a versatile tool with broad applicability. It can be used to annotate individual microbial genomes, including probiotics, as well as metagenome-assembled genomes, providing valuable insights for both targeted studies of specific microbes and community-level functional analyses. This capability is particularly valuable for microbiome research, where understanding the functional roles of both individual species and entire communities is essential.

When tested with *Lp. plantarum* IS-10506 complete genome [5], KEGGaNOG reliably identified key metabolic pathways, including those central to carbohydrate metabolism and short-chain fatty acid synthesis, which are critical to gut health research. Its visualization capabilities greatly enhanced the interpretability of results, enabling researchers to quickly and effectively translate genomic data into actionable biological insights.

KEGGaNOG's user-friendly manual [5], local functionality, and open-source nature make it a versatile and accessible tool for researchers of varying expertise levels. By bridging the gap between eggNOG-mapper and KEGG-Decoder, KEGGaNOG provides universal access to



KEGG pathway annotations and visualizations, fostering new opportunities in gut microbiome and microbial ecology research. Future updates aim to expand compatibility with additional annotation tools and enhance visualization features to further broaden its utility.

References

- M. Shaffer et al., “DRAM for distilling microbial metabolism to automate the curation of microbiome function,” *Nucleic Acids Res.*, vol. 48, no. 16, pp. 8883–8900, Sep. 2020, doi: 10.1093/nar/gkaa621.
- C. P. Cantalapiedra, A. Hernández-Plaza, I. Letunic, P. Bork, and J. Huerta-Cepas, “eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale,” *Mol. Biol. Evol.*, vol. 38, no. 12, pp.
- E. D. Graham, J. F. Heidelberg, and B. J. Tully, “Potential for primary productivity in a globally-distributed bacterial phototroph,” *ISME J.*, vol. 12, no. 7, pp. 1861–1866, Jul. 2018, doi: 10.1038/s41396-018-0091-3.
- F. Mölder et al., “Sustainable data analysis with Snakemake,” Apr. 19, 2021, *F1000Research*: 10:33. doi: 10.12688/f1000research.29032.2.

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PP035: Beneficial and safety properties of bacteriocinogenic lactic acid bacteria obtained from Bulgarian feta cheeses

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The beneficial effects of selected bacteria on human and animal nutrition have been recognized for a long time, with traditional medicine even suggesting the use of numerous fermented products. Cheese are one of the traditional dairy products, where lactic acid bacteria (LAB) plays an essential role in the fermentation, biopreservation, health promoting and organoleptic properties. Moreover, cheese can be regarded as vector for delivery of probiotics to the consumers. Certain strains, part of the commercial microbiota of the cheese, have the ability to produce bacteriocins, which help control undesirable bacterial cultures. Additionally, assessing the safety profile of these strains is a crucial step in selecting them for evaluation as starter, biopreservation or probiotics.

Focus of current study was to evaluate safety profile of *Pediococcus pentosaceus* ST408KOC, *Pediococcus pentosaceus* ST401KOC and *Lactiplantibacillus plantarum* ST414KOC all of them isolated from Bulgarian feta cheese, part of the collection of ProBacLab, University of Sao Paulo, Brazil. In the recent study we have evaluated dynamic of the bacteriocin production for *P. pentosaceus* ST408KOC, *P. pentosaceus* ST401KOC and *Lpb. plantarum* ST414KOC, cultured at 37°C for 24h in MRS broth. Moreover, dynamic of the effect of bacteriocins produced by *P. pentosaceus* ST408KOC, *P. pentosaceus* ST401KOC and *Lpb. plantarum* ST414KOC versus *Listeria monocytogenes* 603 was evaluated over 24h. All tested strains were characterized as gelatinase negative and γ -hemolytic and antibiotic susceptibility/resistance profiles when cultured at 37°C was recorded. Moreover, when exposed to presence of the conditions simulating gastrointestinal conditions, cultured at 37°C the stability and survival were observed. For *P. pentosaceus* ST408KOC when exposed to stomach conditions viable cells were reduced from 8.86×10^8 CFU/ml for 8.94×10^8 CFU/ml and further after lower GIT conditions bacterial titer was 3.36×10^8 CFU/ml. Similar results were observed for *P. pentosaceus* ST401KOC, from 7.56×10^8 CFU/ml to 8.0×10^3 CFU/ml and further to < 100 CFU/ml. When *Lpb. plantarum* ST414KOC was tested, the results were 8.60×10^{10} CFU/ml to 1.33×10^9 CFU/ml and further to 1.20×10^9 CFU/ml, respectively initial microbial load, after stomach and lowered GIT environment. Enzymatic productions recorded for the *P. pentosaceus* ST408KOC pointed expression of leucine and cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase and β -glucosidase, for *P. pentosaceus* ST401KOC was leucine, valine and cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, β -glucosidase and N-acetyl- β -glucosaminidase and for *Lpb. plantarum* ST414KOC was alkaline phosphatase, esterase (C4), leucine, valine and cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -



galactosidase, α -glucosidase, β -glucosidase and N-acetyl- β -glucosaminidase. Studied strains were presenting stability and microbial growth in initial pH between 6.0 and 10.0, presence of oxbile 0.1% or NaCl below 5.0 %. Moreover, based on performed PCR was shown that *P. pentosaceus* ST408KOC, *P. pentosaceus* ST401KOC and *Lpb. plantarum* ST414KOC were negative regarding presence of *gel*, *asa* and vancomycin resistance genes.

It is essential to ensure that the new strains do not pose any health risks to consumers, such as causing infections or adverse reactions. Moreover, safety testing helps confirm that the probiotics do not carry harmful genes, such as antibiotic resistance genes, which could be transferred to pathogenic bacteria.

References

Choi GH, Holzapfel WH, Todorov SD. Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit Rev Microbiol*. 2023 Sep;49(5):578-597. doi: 10.1080/1040841X.2022.2141111

Fugaban JII, Vazquez Bucheli JE, Kim B, Holzapfel WH, Todorov SD. Safety and beneficial properties of bacteriocinogenic *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolated from silage. *Lett Appl Microbiol*. 2021 Dec;73(6):725-734. doi: 10.1111/la

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PP036: Characterization of *Lacticaseibacillus paracasei* ST0110KOC as putative probiotic in healthy human body and hyperthermia conditions

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The utilization of beneficial bacteria in human nutrition has a long-established history, and today, these beneficial effects are recognized as probiotics. A distinctive feature of certain strains is their ability to produce bacteriocins, which can be harnessed to control pathogenic bacteria. Furthermore, the safety profile of probiotic strains is a critical factor in their selection for potential therapeutic use.

This study aims to investigate the impact of body temperature under normal conditions (37°C) and hyperthermic conditions (39°C) on the growth, safety properties, and bacteriocin production of *Lacticaseibacillus paracasei* ST0110KOC, a strain part of the collection ProBacLab, Laboratory of Food Microbiology, Faculty of Pharmaceutical Sciences, University of São Paulo, Brazil. Growth of *Lbs. paracasei* ST0110KOC at 37°C and 39°C in MRS broth adjusted to different pH levels (2.0, 4.0, 6.0, 8.0, 10.0, and 12.0), was pointed as optimal pH range between 6 and 10 at both temperatures. Moreover, when *Lbs. paracasei* ST0110KOC was cultured in the presence of 0.1% and 10.0% bile salt concentrations and variations of NaCl concentrations (0.5%, 1%, 2.0%, 3.0%, 5.0%, and 10.0%), higher concentration of bile salt and NaCl higher than 5.0% were inhibited tested strain. Exposed to the *in vitro* GIT simulation model, the strain exhibited superior growth at 37°C, with a reduction from 2.5×10^9 to 2.97×10^6 , while at 39°C, the decrease was further potentiated, from 2.5×10^9 to 2.04×10^5 . Bacteriocin activity of *Lbs. paracasei* ST0110KOC was 51200 AU/mL tested versus *Listeria monocytogenes* 103, 302 and 408. When *L. monocytogenes* 103 was grown in presence of cell free supernatant containing bacteriocin produced by strain ST0110KOC, similar inhibitory mode of action at both temperatures were observed for the first 12h, however, at 24h *L. monocytogenes* presented slight improvement in recovery when cultured at 39°C. Concerning safety properties, *Lbs. paracasei* ST0110KOC was γ -hemolytic, non-producer of gelatinase (none via physiological, neither presence of the *gel* gene), and non-producer of biogenic amines (histamine, putrescine, and tyramine). Moreover, *ace* (angiotensin I converting enzyme) was not detected in DNA from *Lbs paracasei* ST0110KOC. Enzymatic productions recorded for the *Lbs. paracasei* ST0110KOC pointed expression of leucine, valine and cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, β -glucosidase and N-acetyl- β -glucosaminidase. The β -galactosidase was not expressed in both temperatures. In the antibiogram, the strain exhibited greater resistance to erythromycin at 39°C and to tylosin at 37°C. These findings suggest that temperature may influence critical factors for the use of this strain as a probiotic. This highlights the importance of further research to evaluate its potential use in humans and other animals with varying body temperatures. Understanding the behavior



of probiotic strains under different thermal conditions will contribute to optimizing their therapeutic application, ensuring improved effectiveness and safety in clinical settings.

References

Colombo M, Nero LA, Todorov SD. Safety profiles of beneficial lactic acid bacteria isolated from dairy systems. *Brazilian Journal of Microbiology*, 51, 787-795, 2020.

Fugaban JII, Vazquez Bucheli JE, Kim B, Holzapfel WH, Todorov SD. Safety and beneficial properties of bacteriocinogenic *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolated from silage. *Lett Appl Microbiol*. 2021 73(6):725-734. doi: 10.1111/la

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PP037: Safety and beneficial properties of bacteriocinogenic *Lactococcus lactis* and *Pediococcus pentosaceus* strains with activity against oral cavity related and antibiotic-resistant pathogens

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Bacteriocins are antimicrobial peptides produced by bacteria that have significant potential in pharmaceuticals and bio preservation. In the pharmaceutical industry, bacteriocins offer a promising alternative to traditional antibiotics, especially in the fight against antibiotic-resistant bacteria. Their targeted mode of action reduces the risk of pathogens and spoilage, but with minimal effect on the beneficial microorganisms, making them a safer option for treating infections. Additionally, bacteriocins can be engineered to enhance their stability and efficacy, broadening their therapeutic applications.

In bio preservation, bacteriocins play a crucial role in extending the shelf life of food products by inhibiting the growth of spoilage and pathogenic microorganisms. Their natural origin and effectiveness at low concentrations make them an attractive choice for maintaining food safety and quality without relying on synthetic preservatives. Overall, the use of bacteriocins in these fields not only promotes health and safety but also supports sustainable and eco-friendly practices.

Previously isolated from kimchi and banana and identified as bacteriocinogenic strains, *Pediococcus pentosaceus* 732, *Lactococcus lactis* subsp. *lactis* 431 and *Lactococcus lactis* 808 were evaluated for their safety and beneficial properties, including *in vitro* inhibition of *Listeria monocytogenes* ATCC 15313 and *Staphylococcus simulans* KACC 13241 and *Staphylococcus auricularis* KACC 13252, respectively. According to the performed physiological, biochemical, and biomolecular PCR analysis, *Pd. pentosaceus* 732, *Lc. lactis* subsp. *lactis* 431 and *Lc. lactis* 808 can be considered as safe, since none of the examined virulence genes was detected in the DNA of the studied LAB. However, only *gad* gene associated with GABA production was recorded in DNA isolated from *Lc. lactis* 808 and *Lc. lactis* subsp. *lactis* 431 strains. All tested LAB were γ -hemolysins and non-producers of gelatinase and biogenic amines, which suggested their safety potential. Also, they were relatively susceptible to antibiotics except for streptomycin, tobramycin, and vancomycin for *Pd. pentosaceus* 732. The growth of *Pd. pentosaceus* 732, *Lc. lactis* subsp. *lactis* 431 and *Lc. lactis* 808 and their survival were not significantly affected by ox bile (up to a maximum of 3%) and pH (except pH 2.0 and



4.0). Also, the examined LABs were not inhibited by various commercial extracts as well as most of the tested medications tested in current study. They did not produce proteolytic enzymes but D/L-lactic acid and β -galactosidase. They were designated as hydrophilic. Furthermore, their survivability in artificial saliva, gastric simulation, and enteric passage was measured and followed by the challenge test to evaluate their ability to inhibit the selected oral pathogens in the oral saliva model conditions.

References

Choi, G.-H., Holzapfel, W.H., Todorov, S.D. (2023). Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Critical Reviews in Microbiology*. 49. (05). 578-597.

Choi, G.-H., Fugaban, J.I.I., Dioso, C.M., Bucheli, J.E.V., Holzapfel, W.H., Todorov, S.D. (2021). Selection of bacteriocinogenic *Bacillus* spp. from traditional fermented Korean food products with additional beneficial properties. *Fermentation* 7(4), 271.

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PP038: Isolation and identification of probiotics – study of commercial products

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This study analyzed the viability and microbiological diversity of probiotic strains in commercial products, focusing on their biochemical, genetic and functional characteristics, in addition to evaluating antimicrobial safety. The original packaging of the products indicated CFU counts as: A, B, C, D and F with 5 billion CFU and E with 100 million CFU/capsule, corresponding to 9.70 log CFU/capsule for products with 5 billion CFU. Based on the comparative analysis of the experimental data and values described on the packaging, only products E and F maintained counts within the expected values. Biochemical tests indicated that all strains presented Gram-positive and catalase-negative characteristics, with a predominant morphology of short rods. In the gas production test, the strains of products C, D and E were characterized as producers, while product F did not show gas production activity, considering the microorganism as homofermentative. In product A, gas production varied between strains, suggesting the presence of different microorganisms. The analysis of genetic profiles by rep-PCR demonstrated homogeneity among the strains isolated from products E, F, C and D; in contrast, product A showed varied band patterns, evidencing microbiological heterogeneity. Molecular identification based on sequencing of the 16S rRNA gene allowed the identification of the strains present in the analyzed products. Strains of *Limosilactobacillus reuteri* were identified in products E, C and D, while *Lactobacillus rhamnosus* was predominant in product F. Product A, however, presented a mixed profile composed of *Limosilactobacillus reuteri*, *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus*. Proteolytic activity analyses indicated that *Lbs. rhamnosus* IS01F, *Lpl. plantarum* IS01A and IS07A, and *Lbm. reuteri* IS05A demonstrated proteolytic capacity in skim milk. This activity may represent an advantageous or virulence factor, depending on its application.

In addition, the diacetyl production capacity was investigated, as it is considered a desirable property for probiotic cultures and fermentation, due to its antimicrobial properties and contribution to the typical butter aroma. In the present study, the strains *Lbs. rhamnosus* IS01F, *Lpl. plantarum* IS01A and IS07A, *Lab. acidophilus* IS12A, and *Lmb. reuteri* IS15A were identified as diacetyl producers. The agar diffusion method was used to assess antimicrobial resistance, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. All strains showed susceptibility to the antibiotics recommended by EFSA, except for vancomycin, which is considered intrinsic in lactobacilli. However, it was noted that *Lmb. reuteri* IS01D showed sensitivity to vancomycin. Furthermore, resistance to kanamycin was recorded in *Lpl. plantarum* IS01A and *Lmb. reuteri* IS05A. Antimicrobial resistance in probiotics raises questions about the safety of these microorganisms, since resistance genes can potentially be transferred to pathogenic microorganisms. The results obtained provide a



detailed characterization of commercially available probiotic strains, evidencing both microbiological variability and the implications of safety and efficacy for food and therapeutic applications.

References

Hill, C., Guarner, F., Reid, G. et al. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews of Gastroenterology and Hepatology*. 11, 506&ndas

Huys G, Botteldoorn N, Delvigne F, De Vuyst L, Heyndrickx M, Pot B, Dubois JJ, Daube G. 2013. Microbial characterization of probiotics--advisory report of the Working Group \"8651 Probiotics\" of the Belgian Superior Health Council (SHC). *Molecu*

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PP039: Exploring potential of *Lactiplantibacillus plantarum* ST0414 as a potential bio-protective culture for control of *Listeria monocytogenes*

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Lactic acid bacteria are known for their production of antimicrobial compounds, including bacteriocins, defined as ribosomal synthesized polypeptides or polypeptide complexes usually antagonistic to genetically closely related organisms. In this context *Lactiplantibacillus plantarum* bacteriocins are one of the well-studied antimicrobials, crucial in controlling *Listeria monocytogenes*, a significant foodborne pathogen (Choi et al., 2023). Plantaricins exhibit strong inhibitory effects against *L. monocytogenes* by disrupting its cell membrane, leading to cell death (Arena et al., 2016). This makes them highly effective in enhancing food safety and extending shelf life without relying on synthetic preservatives (Choi et al., 2023). Additionally, *Lpb. plantarum* bacteriocins are natural and safe for human consumption, aligning with the growing consumer demand for natural food additives (Camargo et al., 2018). *Lpb. plantarum* is a well-studied bacteriocinogenic species known for producing diverse bacteriocins, able to inhibit the growth of foodborne pathogens and spoilage bacteria, enhancing food safety and extending shelf life. *Lpb. plantarum* is widely used in fermented foods, such as dairy and meat products, due to its ability to produce bacteriocins and can play beneficial role in enhancing food safety. Representatives from this species also contributes to the sensory qualities of foods and has probiotic properties, promoting gut health. Its application in food preservation and health benefits makes it a valuable microorganism in the food industry. Their application in food preservation helps prevent listeriosis outbreaks, ensuring public health protection. Moreover, the use of these bacteriocins supports sustainable practices by reducing the need for chemical preservatives, thus promoting eco-friendly food production (Arena et al., 2016). Overall, *Lpb. plantarum* bacteriocins play a vital role in both food safety and sustainability.

Lpb. plantarum ST0414 was isolated from Bulgarian white brine cheese produced by medium scale producer and identified based on its biochemical and genetic characteristics including 16S rRNA sequencing. Produced by *Lpb. plantarum* ST0414 bacteriocin was stable when exposed to the pH levels between 2.0 and 10.0, temperatures between 10°C and 121°C and chemicals used in dairy industry. Based on performed biochemical and physiological test, hemolytic, gelatinase, proteolytic and lipolytic activity, biogenic amines, and antibiotic resistance, *Lpb. plantarum* ST0414 can be considered as safe strain. Moreover, the studied strain did not show presence of virulence genes, including *vanA*, B, C, D, E and G and genes associated with biogenic amines production. When *Listeria monocytogenes* L211 was used a sensitive strain, activity of expressed by *Lpb. plantarum* ST0414 of 51200 AU/ml was recorded during stationary growth phase. The addition of 1600 AU/ml bacteriocin produced by *Lpb. plantarum* ST0414 (pH 6.0) to a 3-h-old culture of *L. monocytogenes* L211 (OD_{600nm} 0.293) resulted in growth inhibition for 12 h, suggesting that the mode of activity of studied



bacteriocin is bactericidal. In accordance with obtained results, we can suggest that bacteriocin-producing strain of *Lpb. plantarum* ST0414 can be considered safe and can be used for the control of *L. monocytoses* in the fermented dairy products.

References

- Arena MP, Silvain A, Normanno G, Grieco F, Drider D, Spano G, Fiocco D. Use of *Lactobacillus plantarum* Strains as a Bio-Control Strategy against Food-Borne Pathogenic Microorganisms. *Front Microbiol.* 2016 Apr 13;7:464. doi: 10.3389/fmicb.2016.00464.
- Camargo AC, Todorov SD, Chihib NE, Drider D, Nero LA. Lactic Acid Bacteria (LAB) and Their Bacteriocins as Alternative Biotechnological Tools to Control *Listeria monocytogenes* Biofilms in Food Processing Facilities. *Mol Biotechnol.* 2018 Sep;60(9):712-726.
- Choi GH, Holzapfel WH, Todorov SD. Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit Rev Microbiol.* 2023 Sep;49(5):578-597. doi: 10.1080/1040841X.2022.2

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PP040: Safety and beneficial properties of *Lactococcus* and *Leuconostoc* isolated from conventional and organic artisanal cheeses from Serra da Canastra region, Minas Gerais state, Brazil.

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Canastra Cheese is an artisanal product made from raw milk by small producers in Serra da Canastra region, in Brazil. The production process employs back-sloping inoculation driving fermentation by an endogenous culture called "pingo", which is originated from the whey collected from the previous day's production. Cheeses are dairy products, where lactic acid bacteria (LAB), including those from the genera *Lactococcus* and *Leuconostoc*, play an essential role in fermentation processes as starter and/or adjunct cultures, and contribute as safeguard against pathogens due to their ability to produce numerous antimicrobial metabolites. Although several LAB are generally recognized as safe (GRAS), assessing their safety at the strain level is crucial for their use in the production of fermented foods, including dairy products. In the current study, we have evaluated the safety and beneficial properties for seven LAB strains of *Lactococcus lactis* (1B2, 1B3, 6D5 and 1E4), *Leuconostoc citrium* (10C2) and *Leuconostoc mesenteroides* (13B4 and 13A1) identified based on 16S rRNA partial gene sequence from dairy samples obtained from Serra da Canastra including *pingo*, conventional and organic artisanal cheeses with 1 and 14 days of ripening. As initial criteria for the selection of safe LAB, we evaluated the hemolytic properties, mucin degradation and antibiotic susceptibility/resistance according to EFSA recommendations. In the following step, the strains were evaluated by PCR for the presence of genes associated with vancomycin resistance (*vanA*, *vanB*, *vanC*), biogenic amines production (histidine decarboxylase, *hdc*; tyrosine decarboxylase, *tdc*; ornithine decarboxylase, *odc*) and virulence factors (enterococcal surface protein, *esp*; gelatinase, *gel*; aggregation substance, *asa*; cytolysin, *cyt*; hyaluronidase, *hyl*; endocarditis antigen, *efa*; and enterococcal pathogenicity island, *isl6*). Desirable genes, related to the production of gamma-aminobutyric acid (GABA; *gad*) and nisin-encoding gene (*nis*), as well as the *in vitro* production of diacetyl were also evaluated. All analyzed strains exhibited gamma hemolytic activity, absence of mucin degradation, and sensitivity to antibiotics. None of the tested strains presented genes related to vancomycin resistance, biogenic amine production, or other virulence genes, except for the *Lc. lactis* strains 1E4 and 1B3, which harbored the *esp* gene and *isl6*, respectively. One strain (*Lc. lactis* 6D5) exhibited the *gad* gene, while none of the strains showed the *nis* gene. Furthermore, the same strain (*Lc. lactis* 6D5) displayed low diacetyl production, while the others showed no detectable diacetyl production. Despite some undesirable observed traits, most of the strains of *Lactococcus* and *Leuconostoc*



collected from conventional and organic systems were deemed safe, affirming their suitability for application in food fermentation processes.

References

FUGABAN, J. I. I. et al. Characterization of Partially Purified Bacteriocins Produced by *Enterococcus faecium* Strains Isolated from Soybean Paste Active Against *Listeria* spp. and Vancomycin-Resistant Enterococci. *Microorganisms*, v. 9, n. 5, p. 1085, 2021.

ABE, F. et al. Safety evaluation of probiotic bifidobacteria by analysis of mucin degradation activity and translocation ability. *Anaerobe*, v. 16, n. 2, p. 131–136, 2010.

CHARTERIS, W. P. et al. Antibiotic Susceptibility of Potentially Probiotic *Lactobacillus* Species. *Journal of Food Protection*, v. 61, n. 12, p. 1636–1643, 1998.

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PP041: Safety and beneficial properties of bacteriocinogenic and putative probiotic strain *Latilactobacillus sakei* subsp. *sakei* 2a

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Representatives from the species *Latilactobacillus sakei* are known for their beneficial probiotic properties and health benefits. Found naturally in fermented foods and the human gut, *Ltb. sakei* plays a crucial role in maintaining a balanced intestinal microbiota. This balance is essential for proper digestion, nutrient absorption, and overall gut health. Strains belong to the species *Ltb. sakei* has been shown to inhibit the growth of pathogens and spoilage bacterial species, thereby reducing the risk of infections and promoting a healthy immune system. Additionally, for some strains belong to the species, anti-inflammatory properties that can help alleviate symptoms of gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) were suggested. The use of strains belong to the species of *Ltb. sakei* as a probiotic supplement supports digestive health, enhances immune function, and contributes to overall well-being. Its natural origin and safety profile make it an excellent choice for those seeking to improve their gut health through probiotics.

In current work, we have investigated some of the probiotic features and safety of the bacteriocin-producing *Latilactobacillus sakei* subsp. *sakei* 2a isolated from *linguça*, a traditional Brazilian sausage.

The effect of selected commercial drugs from different generic groups and antibiotics on the growth of *Ltb. sakei* subsp. *sakei* 2a was also determined. The presence of virulence factors was determined based on PCR with total DNA from *Ltb. sakei* subsp. *sakei* 2a. Good growth of *Ltb. sakei* subsp. *sakei* 2a was recorded in MRS broth supplemented with 0.2% or 0.4% oxbile or in MRS broth adjusted to a pH from 5.0–9.0. Auto-aggregation of *Ltb. sakei* subsp. *sakei* 2a was 62.59%. Different levels of co-aggregation were recorded between *Ltb. sakei* subsp. *sakei* 2a and *Enterococcus faecalis* ATCC19443, *Ltb. sakei* ATCC15521 and *Listeria monocytogenes* ScottA. Growth of *Ltb. sakei* subsp. *sakei* 2a was not inhibited by commercial drugs from different generic groups. The inhibitory effect on the growth of *Ltb. sakei* subsp. *sakei* 2a was recorded only in the presence of Arotin [selective serotonin reuptake inhibitor antidepressant] Minimal Inhibition Concentration (MIC) 1.0 mg/mL, Atlansil [Antiarrhythmic] MIC 0.625 mg/mL, Diclofenac potassium [non-steroidal anti-inflammatory drug (NSAID)] MIC 2.5 mg/mL and Spidufen [NSAID] MIC 15.0 mg/mL. Only two antibiotics tested in this study, Amoxil and Urotrobel, inhibited the growth of *Ltb. sakei* subsp. *sakei* 2a with a MIC of



< 0.5 mg/mL and 5.0 mg/mL, respectively. However, *Ltb. sakei* subsp. *sakei* 2a generated positive PCR results on the DNA level for *vanA* (vancomycin resistance), *hyl* (hyaluronidase), *esp* (enterococcal surface protein), *ace* (adhesion of collagen) and *cilA* (cytolysin) and a high virulence profile when examined for the presence of virulence factors. It is important to underline that cytolysis has been described as a virulence and antibacterial factor.

References

Camargo AC, Todorov SD, Chihib NE, Drider D, Nero LA. Lactic Acid Bacteria (LAB) and Their Bacteriocins as Alternative Biotechnological Tools to Control *Listeria monocytogenes* Biofilms in Food Processing Facilities. *Mol Biotechnol.* 2018 Sep;60(9):712-726.

Choi GH, Holzapfel WH, Todorov SD. Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit Rev Microbiol.* 2023 Sep;49(5):578-597. doi: 10.1080/1040841X.2022.2

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PP042: Bacteriocinogenic properties of *Enterococcus faecium* strains isolated from bats fecal samples

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Bats are unique mammals, adapted to specific ecological. Even been mammals, the specificity of the bats GIT microbiota is more like that of the avians, which is dominated by Proteobacteria, most probably related to their lifestyle and inhabitants. As an adaptation, bats have a rapid GIT transit time, which may reduce the stability of their microbiome, that may limit nutrient uptake, and influence pathogen exposure and the evolution of tolerance mechanisms and all this made their microbiome unique and can be regarded as source for differentiated microbial cultures.

The aim of this study was to screen for bacteriocins producers in the fecal samples of bats, with further objective to study development of potential probiotics with health promoting properties for bats, parts of the rehabilitation and conservation programs.

In the isolation process of LAB from the bats fecal samples applied triple layer approach was applied with objective to preselect bacterial cultures with antimicrobial properties. From more than 90 isolates presenting inhibitory properties observed in the preliminary screening, 6 cultures were selected for the following research, according to the preliminary bacteriocin test against *Listeria monocytogenes* 211, 603 and 620. Based on the preliminary Gram staining and catalase test, and test recommended by Burgey's Manual (de Vos et al., 2009), 6 cultures preselected as potential bacteriocinogenic LAB were differentiated by repPCR on total DNA, and two grouped into 2 clusters and further 16s rRNA identification showed to be part of the *Enterococcus faecium* species. It was interesting that same strains were obtained from fecal samples from different animals, pointing that animals from same groups can share their microbial populations.

The selected for further studies *E. faecium* TL01 and TL76 strains were producing antimicrobial peptides (bacteriocins) most probably different from already reported, since we were not able to record presence of tested Enterocin A, B, P, 50 genes. Produced bacteriocins were stable at large margins of pH and temperature, included at 121°C for 15 min and presence of different chemicals applied in food processing processes and analytical procedures. Highest levels of bacteriocins produced by *E. faecium* TL01 and TL76 was recorded during stationary phase (12800 AU/ml and 25600 AU/ml, respectively) when cultured in MRS both at 37°C.



Moreover, the growth inhibition of *L. monocytogenes* 211, 603 and 620 was noted when bacteriocin containing CFS from *E. faecium* TL01 and TL76 was added. Samples collected at 10h from experimental and controls setups showed no presence and around log 8 CFU/ml viable counts, respectively, for *L. monocytogenes* strains. Bacteriocins produced by *E. faecium* TL01 and TL76 were showing very selective inhibitory activity against different strains of *L. monocytogenes* and only a few of other test microorganisms included in the examination panel were inhibited. *E. faecium* TL01 and TL76 can be considered as safe, as performed test showed negative results for the gelatin hydrolysis, proteolytic and hemolytic activity, mucin degradation tests and antibiotic resistance/sensitivity and further screening for presence of some virulence genes (recommended by Fugaban et al., 2021) regarding safety for enterococci.

References

- Choi, G.H., Holzapfel, W.H., Todorov, S.D. (2023). Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Critical Reviews in Microbiology*, 49 (5) 578-597. doi:10
- Fugaban, J.I.I., Holzapfel, W.H., Todorov, S.D. (2021). Probiotic potential and safety assessment of bacteriocinogenic *Enterococcus faecium* strains with antibacterial activity against *Listeria* and vancomycin-resistant enterococci. *Current Research in Microbiology*
- De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., & Whitman, W.B. 2009. *Bergeys manual of systematic bacteriology: the firmicutes*. London: Springer.

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PP043: Evaluation for optimal conditions of γ -aminobutyric acid production by *Lactiplantibacillus plantarum* ST0414

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Lactic acid bacteria are known for their beneficial properties, including production of various antimicrobials and health promoting metabolites, such as γ -aminobutyric acid (GABA), a non-protein amino acid that acts as a neurotransmitter, produced mainly through the decarboxylation of glutamate by the enzyme glutamate decarboxylase. *Lactiplantibacillus plantarum* is a species applied in the fermentation of dairy, meat and plant-based products, where plays an essential role as starter, adjunct, protective or probiotic/postbiotic culture, increasing functional properties in the synthesis of its metabolites, such as GABA. The objective of this study was to evaluate safety and GABA production by *Lpb. plantarum* ST0414 and explore influence of the culturing conditions such as cell density of the producer, concentration of monosodium glutamate (an GABA precursor), pH, temperature and incubation time. Expressed GABA was quantified by gas chromatography coupled to mass spectrometry (GC-MS). In this study we have studied *Lpb. plantarum* ST0414 strains, isolated from Bulgarian white brine cheese produced by medium scale producers and identified based on its biochemical and genetic characteristics including 16S rRNA sequencing. The strain ST0414 was selected for current study based on preliminary screening for GABA production between more than 150 bacterial isolates, most of them obtained from dairy products. GABA production from *Lpb. plantarum* ST0414 was evaluated in different levels of cellular density (10⁵ -10⁸ CFU/ml), monosodium glutamate concentration (30 mM-120 mM), pH (4.0-8.0), temperatures (from 30°C to 50°C) and incubation time (24h-120h), varying one method at a time, maintaining other standard conditions of 10⁶ UFC/ml, 60 mM, pH 6.0, 37°C and 24h and following to additional experimental set-ups. Cell free supernatant was obtained after centrifuged at 15.000×g for 15 minutes at 20°C, diluted 10×, derivatized in triplicate with methyl chloroformate and quantified by GC-MS. Comparing results obtained from the different experiments following preselected variables, the production of GABA by *Lpb. plantarum* ST0414 was present in superior levels when was subjected to a longer incubation time 120h (27.98 mg/ml) and microbial density of 10⁵ CFU/ml (16.13 mg/ml), respectively. Extreme temperatures of 30°C and 50°C used demonstrated better results (13.64 mg/ml and 14.27 mg/ml, respectively), being assimilated to the greater production of GABA under stress conditions by the ST0414 strain. Variations in pH and glutamate concentration did not prove to be influencing factors in the production of GABA, maintaining close values between their respective variations. Moreover, based on performed biochemical and physiological tests, hemolytic, gelatinase, proteolytic and lipolytic activity, biogenic amines production and antibiotic resistance, including different van genes, *Lpb. plantarum* ST0414 can be considered as a safe



strain. Obtained results, suggest that GABA production can be increased when *Lpb. plantarum* ST0414 is exposed to stress growth conditions. In current study we have recorded that *Lpb. plantarum* ST0414 has optimal conditions for GABA production with 120h of incubation, cell density of 105 UFC/ml and temperature of 30°C or 50°C. Thus, information can be further applied and scaled with aim of biotechnological production where *Lpb. plantarum* ST0414 can be explored as industrial GABA producer.

References

RAWLINSON, C. et al. A rapid method for profiling of volatile and semi-volatile phytohormones using methyl chloroformate derivatization and GC–MS. *Metabolomics*, v. 11, n. 6, p. 1922–1933, 1 dez. 2015.

ZHANG, Q. et al. Characterization of γ -aminobutyric acid (GABA)-producing *Saccharomyces cerevisiae* and coculture with *Lactobacillus plantarum* for mulberry beverage brewing. *Journal of Bioscience and Bioengineering*, v. 129, n. 4, p. 447–453,

ZHUANG, K. et al. Transcriptomic response to GABA-producing *Lactobacillus plantarum* CGMCC 1.2437T induced by L-MSG. *PLOS ONE*, v. 13, n. 6, 1 jun. 2018.

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PP044: Screening for lactic acid bacteria with bacteriocinogenic potential from Brazilian artisanal and indigenes fermented food products

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Lactic acid bacteria (LAB) were historically recognized as important microorganisms used in the fermentation of dairy, meat, and vegetable products, playing a significant role in the development of flavors and textures, contributing to the safety and even can be considered as probiotics or postbiotics with health promoting properties for the consumers. As part of the metabolic properties, LAB can produce various antimicrobials, including bacteriocins, which provide a competitive advantage in interactions with other microorganisms. Bacteriocins are protein compounds that exhibit bactericidal or bacteriostatic activity against genetically related strains, including spoilage, foodborne and clinically relevant pathogens.

This study aimed to isolate bacteriocin-producing LAB strains from various artisanal and indigenous fermented foods products from Brazilian territories. For the isolation, the samples were homogenized in saline solution (0.85% NaCl), subjected to serial dilutions, and plated on Man, Rogosa, and Sharpe (MRS) agar, followed by incubation at 37 °C for 48 hours. The obtained colonies were cultivated in MRS broth at 37 °C for 24 - 48 hours and evaluated via biochemical and morphological tests for identification and classified as LAB based on catalase-negative and Gram-positive results (de Vos et al., 2009). The selected strains were evaluated for bacteriocin production against seven strains of *Listeria monocytogenes* and three strains of *Staphylococcus aureus*. For this, cell-free supernatants were collected by centrifugation (10.000 ×g for 10 min at 20°C) from previously grown cultures in MRS broth for 24h at 37°C. The supernatants were heat-inactivated (80°C for 10 min) and tested using the spot-on-the-lawn technique on BHI agar plates containing target strains. Bacteriocin activity was determined by the formation of inhibition halos around the treated supernatants (Fugaban et al., 2021).

Samples from numerous products were analyzed, including water kefir, milk kefir, sourdough starter, probiotic curd, fermented cassava flour, fermented cashew nut paste with olives, kombuchas of different flavors and brands, fresh Minas cheese, and fermented kefir and apple beverage. In total, 348 isolates were obtained, of which 123 were identified as LAB. Sourdough starter, probiotic curd, fermented paste, and fresh Minas cheese samples showed the highest proportion of LAB, while kefir, kombucha, and fermented beverage samples were dominated by yeasts, as observed based on physiological and biochemical tests. The antimicrobial activity was stronger when *L. monocytogenes* were applied as test microorganisms, compared to tested *S. aureus* cultures. This study highlights the microbial diversity in Brazilian artisanal fermented foods and the potential of LAB as sources of bacteriocins with antimicrobial activity. The



findings suggest that specific LAB strains could be explored for the development of natural biopreservatives, especially for controlling foodborne pathogens such as *L. monocytogenes*. Future research should focus on the molecular characterization of the selected microbial cultures, their safety and expressed antimicrobials and additional beneficial metabolites and their application in various food matrices as potential beneficial cultures.

References

Fugaban, J.I.I., Bucheli, J.E.V., Kim, B., Holzapfel, W.H., Todorov, S.D., 2021. Safety and beneficial properties of bacteriocinogenic *Pediococcus acidilactici* and *Pediococcus pentosaceus* isolated from silage. *Letters in Applied Microbiology*. 73(6), 725-7

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B., 2009. *Bergeys Manual of Systematic Bacteriology: The Firmicutes*; Springer: London, UK, 2009.

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PP045: *Enterococcus* spp. are predominate bacteriocinogenic strains in Feta Cheese, however, *Pediococcus acidilactici* can be the best candidate for the further biopreservation

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Introduction: Lactic Acid Bacteria (LAB) and their metabolites are promising alternatives to promote food safety, quality assurance and even serve as probiotics and postbiotics. Moreover, food borne pathogens such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* and *Staphylococcus aureus* are some of the most frequent food safety concerns. The crossing point between beneficial and spoilage are some of the metabolites produced by LAB, representing antagonistic activity and their applications as biopreservatives, meeting the growing consumer demand for alternatives to chemical preservatives (Choi et al., 2023).

Objective: The aim of this study was to isolate bacteriocin-producing LAB from traditional fermented food products and evaluate their potential as putative food protective agents.

Results: In the preliminary screening LAB from different samples of dairy origin were evaluated for the bacteriocin production properties. A set of 14 preliminary identified as LAB based on catalase test and Gram staining, were isolated from Bulgarian feta cheese. After the confirmation that production antimicrobials by selected LAB were on proteinaceous nature (bacteriocin/s), the strains were differentiated by repPCR and identified based on recommendations from Burgey's Manual (de Vos et al., 2009) and following 16S rRNA partial gene sequencing. Tested 14 isolates were grouped in 5 different clusters and representative from each groups identified as *Enterococcus faecium* (K33), *Enterococcus faecalis* (K35, K37, K40) and *Pediococcus acidilactici* (K41). The spectrum of activity for the selected 5 strains was evaluated against taxonomically related microorganisms, including different *Listeria monocytogenes* strains and several beneficial strains from the microbial collection of ProBacLab, Laboratory of Food Microbiology, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil. All tested strains represented strong anti-Listerial activity. The studied bacteriocins were shown to be stable to variations in pH, temperature and against chemical compounds used in the food industry (Tween 20 and 80, NaCl, milk and SDS). Selected 5 strains were presented reasonable survive in simulated in vitro conditions of the gastrointestinal system. Moreover, related to the safety assessment experiments, the studied LAB strains demonstrated safety profile, regarding the hemolytic outline and proteolytic and gelatinase activity, which were not detected. The antibiotic resistance/susceptibility based on physiological and biomolecular, and presence of virulence genes were also assessed. It was interesting that two of the isolates exhibited resistance to vancomycin (*Enterococcus faecalis* K40 and *Pediococcus acidilactici* K41) and potential virulence factors were found in some



strains, namely gel (in all), esp (in *Enterococcus faecalis* K40), efa and asa (in *Enterococcus faecium* K33 and *Enterococcus faecalis* K35, K37 and K40).

Conclusion: Compared with the other isolates, *Pediococcus acidilactici* K41 proved to be a good candidate for use as an agent to be incorporated as a biopreservative. However, although the other isolates possess virulence genes, the bacteriocins produced by them can be incorporated, after purification, in food production to prevent the growth of potentially pathogenic microorganisms, such as *Listeria*.

References

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., & Whitman, W.B. 2009. *Bergeys manual of systematic bacteriology: the firmicutes*. London: Springer.

Choi GH, Holzapfel WH, Todorov SD. Diversity of the bacteriocins, their classification and potential applications in combat of antibiotic resistant and clinically relevant pathogens. *Crit Rev Microbiol*. 2023 Sep;49(5):578-597. doi: 10.1080/1040841X.2022.2

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PP046: Isolation of lactic acid bacteria and *Bacillus* spp. from tropical fruits and screening for safe candidates for exploring their beneficial properties

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Lactic acid bacteria (LAB) play a crucial role in the preservation and enhancement of fruits, contributing significantly to food safety, nutritional value, and sensory properties. These beneficial microorganisms are widely recognized for their ability to ferment sugars into lactic acid, which lowers the pH and creates an environment that inhibits the growth of spoilage organisms and pathogens. This natural preservation method extends the shelf life of fruits and fruit products, reducing food waste and ensuring a safer food supply. Moreover, in addition to the preservation properties, LAB also enhance the nutritional profile of fruits by producing bioactive compounds such as vitamins, antioxidants, and antimicrobial peptides. These compounds can improve the health benefits of fruits, making them more appealing to health-conscious consumers. Some LAB can be actively involved in the reduce the content of anti-nutritional factors and allergens in fruits, further enhancing their nutritional value and even can be potential probiotic candidates.

The Brazilian biome is a rich and diverse source of tropical fruits, many of which are little known outside Brazil and Latin America. These fruits are not only appreciated for their gastronomic characteristics but are also used in traditional medicine for their bioactive properties. Exploring the microbiota of these fruits may reveal new strains of bacteriocin-producing bacteria, which have promising therapeutic applications. The project aims to isolate and identify new strains of LAB and *Bacillus* spp. from tropical fruits, evaluate their safety and screen for beneficial properties with aim to be further investigated as putative probiotics.

More than 260 isolates were obtained from banana, Brazilian cheery, lemon, *pitanga*, clementine, pineapple, guava, *atemoya*, *caju* collected in the area of Sao Paulo, SP, Brazil. All fruits were collected with minimal manipulation interference and further macerated in proportion 1:9 with sterile saline. A serial dilutions were prepared and plated on MRS agar and plates incubated for 24-48h in aerobic conditions. Colonies with morphology typical for LAB and *Bacillus* spp. were isolated for further investigation. Morphology was observed after Gram staining and catalase test were performed. Preselection of isolates of interest included tests for hemolytic activity, where 38% were included for further study as γ -hemolytic. Isolates were tested for resistance/susceptibility to antibiotics, recommended by EFSA (ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, streptomycin, tetracycline and vancomycin) and results served to selected non multidrug resistance isolates. In addition, mucin degradation and lipolytic activity were served as criterium for selection of safe strains. Only 54 from 260 isolated, preselected were subject to differentiated by repPCR and further identified according 16S rRNA partial gene sequencing. In addition to LAB



(*Lactiplantibacillus plantarum*, *Levilactobacillus brevis* and *Streptococcus thermophilus*) and *Bacillus* spp. (*Bacillus licheniformis*, *Bacillus aerius* and *Bacillus paralicheniformis*), *Staphylococcus epidermidis*, *Staphylococcus partial*, *Staphylococcus hominis*, *Escherichia coli*, *Escherichia alberti*, *Listeria monocytogenes*, *Kosokinia cowanii* and *Enterobacter cloaca* were identified, a fact that is rising concerns regarding safety of the direct consumption of the not hygienized fruits.

References

De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., & Whitman, W.B. 2009. Bergey's manual of systematic bacteriology: the firmicutes. London: Springer.

Ye, J., Fugaban, J.I.I., Dioso, C.M., Vazquez Bucheli, J.E., Choi, G.-H., Kim, B., Holzapfel, W.H., Todorov, S.D. 2023. *Bacillus* strains isolated from Korean fermented food products with antimicrobial activity against staphylococci, an alternative for con

Acknowledgements: This study was supported by Sao Paulo Research Foundation (FAPESP) (grants 2023/05394-9; 2024/01721-8) and by the Centre for Research and Development in Agrifood Systems and Sustainability, funded by FCT (UIDB/05937/2020 and UIDP/05937/2020), Fundação para a Ciência e a Tecnologia, Portugal.



PP047: Antioxidant properties of *Streptococcus thermophilus*, *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* strains isolated from Brazilian tropical fruits

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Lactic acid bacteria (LAB) are renowned for their antioxidant properties, which play a crucial role in mitigating oxidative stress in the body. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and the body's ability to detoxify these reactive intermediates or repair the resulting damage. Excessive ROS can lead to cellular damage, contributing to various diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. LAB exhibit antioxidant activity through several mechanisms. They produce antioxidant enzymes such as superoxide dismutase (SOD) and can have pseudo-catalase activity, which neutralize ROS. Additionally, LAB can scavenge free radicals directly. For instance, strains like *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum* have demonstrated significant free radical scavenging abilities (Bryukhanov et al., 2022). Moreover, LAB can enhance the body's antioxidant defense by modulating the gut microbiota. They promote the growth of beneficial bacteria that produce short-chain fatty acids (SCFAs), which have anti-inflammatory and antioxidant effects. LAB also produce exopolysaccharides and other metabolites that contribute to their antioxidant capacity.

Streptococcus thermophilus L32, *Lactiplantibacillus plantarum* L3 and *Levilactobacillus brevis* L5 were isolated from Brazilian lemon, differentiated by repPCR and identified by biomolecular analysis, including partial sequencing of 16S rRNA. They were characterized regarding their antioxidant properties. Studied strains can be considered safe, based on the evaluation of hemolytic activity, degradation of mucin, susceptibility/resistance to antibiotics and production of the enzyme gelatinase. In addition, they can also be considered potentially beneficial based on their stability and survival in simulated gastrointestinal tract conditions (stomach and duodenum), β -galactosidase production, diacetyl production and specific levels of hydrophobicity.

Recorded levels of DPPH in the studied strains ranged between 93.52% for *Lpb. plantarum* L3, 77.88% for *Lvb. brevis* L5, to 53.29% for *Str. thermophilus* L32, indicating that antioxidant properties are strain specific, compared to the positive control, ascorbic acid (1 mg/mL) (74.80%). The beneficial attributes of these cultures to fermented food products may enable the reduction of chemical additives in line with consumers' demand for more natural and chemical-free food commodities.

Several studies have evaluated the DPPH antioxidant activity of *Str. thermophilus* with DPPH inhibition values ranging from 18.97% to 44.44%, indicating varying levels of antioxidant activity among the isolates (Kim et al., 2022). *Lpb. plantarum* showed significant DPPH radical



scavenging activity, with some strains achieving scavenging percentages as high as 58.1%, or even comparable to those of ascorbic acid (Martiz et al., 2023). Research has shown that *Lvb. brevis* strains can achieve DPPH inhibition rates of up to 94.47%, making it one of the more effective LAB in terms of antioxidant potential (Won et al., 2021), while in different study was reported DPPH inhibition rates of 60.51% for *Lvb. brevis* (Qin et al., 2024).

The antioxidant properties of the studied strains can be applied to food preservation, reducing or even eliminating the need to use chemical additives, providing healthier options for consumers.

References

- Bryukhanov, A.L. et al. Antioxidant properties of lactic acid bacteria. *Microbiology* 91, 463–478 (2022). <https://doi.org/10.1134/S0026261722601439>
- Kim, S. et al. Antioxidant activity and probiotic properties of lactic acid bacteria. *Fermentation* 8(1), 29 (2022). <https://doi.org/10.3390/fermentation8010029>
- Martiz, R.M. et al. Inhibition of carbohydrate hydrolyzing enzymes by a potential probiotic *Levilactobacillus brevis* RAMULAB isolated from fermented A
- Won, G. et al. In vitro antidiabetic, antioxidant activity, and probiotic activities of *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* strains. *Curr. Microbiol.* 78, 3181–3191 (2021). h

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PP048: Local artisanal dairy fermented products as reservoir of beneficial microorganisms

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Beneficial microorganisms provide added value to food products or hosts and correspond to what is often referred to as "positive flora". These microorganisms exhibit diverse biological activities and produce metabolites of interest, which can positively impact food preservation, flavor development, and host health. Beneficial microorganisms can generally be classified into two main categories: antagonistic microorganisms and probiotic microorganisms.

Antagonistic microorganisms are those that inhibit undesirable microorganisms, by either active or passive competition. Active antagonism involves the production of bioactive molecules, such as bacteriocins, organic acids, or antifungal compounds, which directly suppress harmful organisms. Passive competition, on the other hand, relates to microorganisms occupying ecological niches or competing for nutrients without necessarily producing active molecules. These mechanisms have been extensively studied in bacteria; however, research on yeasts exhibiting similar antagonistic properties remains relatively limited and is a growing area of interest.

Probiotic microorganisms, in contrast, must demonstrate a beneficial biological activity *in vivo* within a host. These microorganisms, including certain bacterial and yeast strains, are capable of promoting gut health, improving immunity, and supporting overall host well-being.

Fermented foods, such as artisanal cheeses, are excellent examples of how beneficial microorganisms can enhance food preservation while simultaneously providing health and sensory benefits. During the ripening process of traditional cheeses, complex microbial ecosystems develop, consisting of bacteria, yeasts and molds. These organisms play a crucial role in flavor development, texture formation and the inhibition of spoilage flora or pathogens.

For example, in the Hauts-de-France region of the north of France, the artisanal cheese Tomme d'Orchies was studied using metagenetic analysis during its ripening process. This analysis revealed the microbial diversity present in both the rind and core of the cheese and highlighted the evolution of bacterial and yeast microbiota over time. Notably, two strains of *Kluyveromyces* isolated from this cheese demonstrated interesting antagonistic activities.

Further studies on other regional cheeses, such as Carré du Vinage and Bourle Ronquoise, identified and isolated antimicrobial strains of yeasts and bacteria. Two specific yeast strains were tested in a curd model, where they demonstrated significant biopreservation potential by



inhibiting spoilage microorganisms. These results suggest that such yeasts could be valuable for extending the shelf life and safety of cheeses.

Fermented foods represent a significant reservoir of beneficial microorganisms with wide-ranging applications. Beyond food biopreservation, these microorganisms have potential uses in improving animal health, human gut health, and even reducing food waste by preventing spoilage. Artisanal cheeses, therefore, serve as both cultural heritage and a valuable platform for exploring microbial diversity and its positive impacts on food systems and health.

References

Commenges A, Lessard MH, Coucheney F, Labrie S, Drider D. 2024. The biopreservative properties of *Metschnikowia pulcherrima* LMA-2038 and *Trichosporon asahii* LMA-810 in a model fresh cheese, are presented. *Food Bioscience*.58: 103458. doi : 10.1016/j.fbio.2

Ceugniez A, Coucheney F, Jacques P, Daube G, Delcenserie V, Drider D. 2017. Anti-Salmonella activity and probiotic trends of *Kluyveromyces marxianus* S-2-05 and *Kluyveromyces lactis* S-3-05 isolated from a French cheese named “Tomme d’Orchie

Acknowledgements:



PP049: Use of metabarcoding for bacterial identification in light and dark pollen from Mandaguari (*Scaptotrigona postica*), a native bee from the Brazilian Cerrado

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Scaptotrigona postica (Mandaguari-black) is a stingless bee (SB) species native to the Brazilian Cerrado, which produces and stores large quantities of fermented pollen (called sambura or bee bread), a protein food that feeds the hive. In addition to proteins, bee bread contains essential amino acids, bioactive compounds, minerals, fatty acids, fiber, microorganisms and bee salivary secretions. Honey and pollen are stored in cerumen pots, a mixture of wax and propolis, that also contain bioactive compounds and microorganisms, which are transferred to the pollen during the storage period and contribute to the fermentation process. These microorganisms, besides establishing a symbiotic relationship with bees and their inputs, assist in the fermentation process of pollen, influencing its maturation, protection against pathogens and development of brood cells. The development of high-throughput techniques has favored the elucidation of microbial communities present in different ecosystems, including food. In this study, bacterial ecology was analyzed using the metabarcoding technique (metataxonomy) by sequencing the 16S rRNA gene of light and dark pollen from Mandaguari. After extracting pollen DNA, molecular identification of microorganisms was performed by preparing 16S ribosomal gene amplicon libraries. The library was sequenced using Illumina® Nextseq™. The DADA2 method was used for bioinformatics, and errors and chimeric sequences were removed. ASV (amplicon sequence variant) were compared with the SILVA v.132 database. Alpha diversity was assessed using estimates from the following indices: Chao1, Simpson, Faith's PD, Evenness, and Shannon. In light pollen, the most frequent phyla identified were Firmicutes and Proteobacteria (Alphaproteobacteria), while in dark pollen, the phyla Firmicutes, Fusobacteriota, Proteobacteria (Alphaproteobacteria and Gammaproteobacteria) were identified. In light pollen, an abundance of the Lactobacillaceae family (> 99%) was observed, with a predominance (> 90%) of the *Lactobacillus* genus. In dark pollen, although the Lactobacillaceae family was also predominant (> 95%), there was greater bacterial diversity (alpha diversity), with a reduction in the abundance of the *Lactobacillus* genus (75%) and an increase in genera little (or not) observed in light pollen, such as *Acetobacter*, *Bombella*, *Phyllobacterium*, *Acinetobacter*, *Fructobacillus*, *Bacillus*, *Erwinia*, *Leuconostoc*. Freshly collected pollen is lighter and less acidic. The causes of pollen darkening include oxidation of phenolic compounds and lipid oxidation. Additionally, during storage, the lactic acid bacteria (LAB) undergo lactic fermentation, with progressive darkening and a decrease in pH due to the production of lactic acid. Microorganisms more attuned to acidic environments, such as different genera of LAB, e.g. *Lactobacillus*, *Leuconostoc* and *Fructobacillus*, participate in biochemical processes that modify the nutritional quality and improve the digestion and absorption of pollen by bees, in addition to stimulating the bees' immune system. Moreover, LAB contributes to the protection of hives from pests and pathogens. The metabarcoding



technique revealed differences in the microbial composition of light and dark pollen, with a greater diversity of bacterial genera in the dark pollen of *S. postica*. However, in both foods there was a predominance of LAB. LAB are very interesting microorganisms for biotechnological applications and the LAB isolated from SB pollen may be promising as probiotics, both for human and bee health.

References

- Alves VF, Chaul LT, Bueno GCA, Reinecke I, Silva TCG, Brito PVA, De Martinis ECP (2024) Associated bacterial microbiota of honey and related products from stingless bees as novel sources of bioactive compounds for biotechnological applications. *COFS*, 55:1
- Sinpoo C, In-on A, Noirungsee N, Attasopa K, Chantawannakul P, Chaimanee V, Phokasem P, Ling TC, Purahong W, Disayathanoowat T. Sinpoo. Microbial community profiling and culturing reveal functional groups of bacteria associated with Thai commercial stingl
- Tang QH, Miao CH, Chen YF, Dong ZX, Cao Z, Liao SQ, Wang JX, Wang ZW, Guo J (2021) The composition of bacteria in gut and beebread of stingless bees (*Apidae: Meliponini*) from tropics Yunnan, China. *Anton Leeuw*, 114(8):1293-1305. doi: 10.1007/s10482-021-01

Acknowledgements: FAPEG, CAPES.



PP050: Adult Zebrafish Model for Potential Probiotics Tissue-Specific Oxidative Stress and Antioxidant Assessment

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The importance of identifying and studying new antioxidant additives is evident in all areas of animal husbandry, including aquaculture. The use of *Danio rerio* to select new antioxidants seems relevant due to its genetic tractability and established use in toxicological studies [1]. This work introduces a model designed to investigate tissue-specific responses to oxidative stress, and subsequently, the effects of antioxidants and probiotics, using adult zebrafish, instead of a common practice of using zebrafish embryos [2]. Such a model will have applications in the development of probiotics and feed additives for aquaculture and beyond. A model of oxidative stress was established in adult zebrafish *D. rerio* to evaluate the antioxidant effect of feed additives. Paraquat, at a final concentration of 10 μ M was used as an inducer of oxidative stress, generating free radicals and providing detectable levels of oxidative stress. Two groups of *D. rerio* were used: wild type (WT) and genetically modified line «GloFish». From each group were formed a control group kept under normal conditions and an experimental group kept in water with paraquat at a concentration of 10 μ M. Oxidative stress was assessed in three tissue types (muscle, intestine, and gills) using two methods: (1) a bioluminescent test on *E. coli* MG1655 pSoxS-lux biosensor to measure antioxidant activity, and (2) quantification of malondialdehyde (MDA), a marker of lipid peroxidation (LPO). Measurements were taken over a 35-day period.

The highest level of antioxidant activity was observed in the intestine of wild-type fish. Exposure to paraquat resulted in increased antioxidant activity in the intestine by the end of the experiment, while it gradually decreased in the absence of paraquat. Conversely, in muscle tissue antioxidant activity gradually decreased by the end of the experiment.

Similar trends were observed in the GloFish group. Specifically, in the presence of paraquat, intestinal antioxidant activity in GloFish decreased to 5% by day 14, then recovered to 25% by day 35. In contrast, muscle tissue showed an increase in protective effect by day 14, followed by a decrease to 35% on day 35. MDA levels were elevated in the muscle and intestine of wild-type *D. rerio* on day 35 of the experiment, indicating increased LPO. The study revealed that wild type *Danio rerio* were more resistant to the effects of paraquat than «GloFish».

A negative correlation was observed between changes in antioxidant activity and the levels of oxidation products, supporting the validity of the model.

To prove the applicability of this model to testing probiotics for aquaculture, we added preparation of two probiotic *Bacillus* strain with confirmed in vitro antioxidant activity. Although probiotic itself did not enhance the survival of fish in normal conditions, it increased survival rate under conditions of oxidative stress by 46.16%.

This study demonstrates the efficacy of combining biochemical assays and biosensor



technologies to comprehensively assess the dynamic interplay between antioxidant and pro-oxidant factors in zebrafish. The findings highlight the tissue-specific and strain-dependent differences in the response to oxidative stress and the negative correlation between antioxidant activity and lipid peroxidation.

References

1. Kari, G., Rodeck, U., & Dicker, A. P. (2007). Zebrafish: an emerging model system for human disease and drug discovery. *Clinical Pharmacology & Therapeutics*, 82(1), 70-80.
2. Mugoni, V., Camporeale, A., & Santoro, M. M. (2014). Analysis of oxidative stress in zebrafish embryos. *Journal of visualized experiments: JoVE*, (89), 51328.

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PP051: Evaluation of effects and genomic analysis of aquaculture probiotic strains with antioxidant and enzymatic activity

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During a screening for *Bacillus* strains with probiotic properties for aquaculture, we isolated five strains with proteolytic and amyolytic activity and five strains with high antioxidant activity. Antioxidant activity was assessed *in vitro* using bacterial LUX-biosensors [1].

Based on tests of cross-antagonistic activity and the ability of strains to form joint biofilms, two pairs of strains that did not inhibit each other's growth during co-cultivation were selected. Probiotic preparations were developed using solid-state fermentation of soybeans. Strains *Bacillus velezensis* MT14 and MT42 (Preparation 1) exhibited proteolytic and amyolytic activity, while strains *Bacillus subtilis* MT48 and MT74 (Preparation 2) demonstrated antioxidant and amyolytic activity.

The preparations were incorporated into starter feeds for sterlet larvae at a concentration of 0.1% of feed weight (final concentration: 10⁶ CFU/g). Fish larvae were fed for two months. Both experimental groups showed weight gain compared to the control group receiving a commercial probiotic. However, the enzymatic probiotic performed better than the antioxidant one. This can be attributed to its proteolytic properties, which aid in protein digestion and absorption which is critical during early development and rapid growth stages.

Interestingly, gene expression analysis in sterlet tissues revealed a more pronounced effect in Group 2 (antioxidant probiotic). The antioxidant probiotic based on strains MT48 and MT74 demonstrated statistically significant protective effects on sterlet larvae. A reduction in *il-1b* gene expression was observed in both experimental groups, indicating reduced inflammation in fish receiving probiotics. Additionally, Group 2 showed decreased expression of *igf-1*, *hsp-70*, and *gst* genes expression. The downregulation of *gst* (associated with oxidative stress response) and *hsp-70* (linked to protein damage response) may suggest a positive impact of the antioxidant probiotic in mitigating stress and damage. Notably, the most significant effects were observed in larvae at the beginning of the experiment, implying that these probiotics are more effective during early developmental stages than in adult fish.

Genomic sequencing of the isolated strains revealed that those with antioxidant activity possessed genes responsible for pulcherriminic acid synthesis, absent in other strains. This



suggests that pulcherriminic acid may act as an intermediate contributing to the antioxidant properties of these strains. Pulcherriminic acid is known for its antioxidant potential and is commonly found among *Bacillus* species [2]. Furthermore, all analyzed strains carried genes for bacilysin and bacillaene synthesis, and for a synthesis of variety of non-ribosomal products.

This study highlights the potential of *Bacillus*-based probiotics for aquaculture applications, demonstrating their ability to enhance growth performance and provide protective effects against oxidative stress in sterlet larvae. Our findings also emphasize the importance of strain-specific properties when developing probiotics tailored to aquaculture needs.

References

1. Bazhenov, S. V., Novoyatlova, U. S., Scheglova, E. S., Prazdnova, E. V., Mazanko, M. S., Kessenikh, A. G., ... & Manukhov, I. V. (2023). Bacterial lux-biosensors: Constructing, applications, and prospects. *Biosensors and Bioelectronics*: X, 13, 1003
2. Angelini, L. L., Dos Santos, R. A. C., Fox, G., Paruthiyil, S., Gozzi, K., Shemesh, M., & Chai, Y. (2023). Pulcherrimin protects *Bacillus subtilis* against oxidative stress during biofilm development. *npj Biofilms and Microbiomes*, 9(1), 50.

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PP052: Functional Minas Frescal cheese with spore-forming *Weizmannia coagulans* GBI-30

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The effect of the addition of *Weizmannia coagulans* BC30 on the probiotic survival and tolerance in the gastrointestinal tract, the anti-pathogenic activity along the storage time (1, 7, 14 days, spot diffusion antimicrobial susceptibility test) and the generation of bioactive compounds (antioxidant, antihypertensive and antidiabetic values) considering three different dosages (6-7, 8-9, 10-11 log CFU/g, respectively, QII, QIII, QIV) was studied. For comparison, a conventional cheese was also produced without the addition of *W. coagulans* BC30 (QI). Pathogenic strains of *Escherichia coli* ATCC 25922, *Listeria monocytogenes* ATCC 19,117 and *Salmonella enterica* subsp. *diarizonae* ATCC 12,325, all from clinical or dairy isolates, were used to evaluate the antibacterial activity of the probiotic cheeses. The non-pathogenic strain *Listeria innocua*, a surrogate for *Listeria monocytogenes*, was also used. *W. coagulans* showed good survival (>6 log UFC/g) and tolerance to gastrointestinal disorders throughout the storage period. The probiotic count after passing through the GIT conditions fluctuated between 6.12 and 6.71, 7.34–7.89, and 8.12–8.56 log CFU g⁻¹ for QI, QII, and QIII, respectively, proving to be constant and proportional to the concentration of probiotics added to the samples. This observation confirms that Minas Frescal cheese is an excellent food matrix for maintaining probiotic viability and protecting against adverse conditions in the gastrointestinal tract. The antioxidant activity (DPPH), antihypertensive activity (angiotensin-converting enzyme inhibitory activity, ACEi), antidiabetic activity (α -amylase and α -glucosidase, and proteolytic activity showed a similar behavior over the storage time of the probiotic cheeses, with higher inoculation concentrations generating a correspondingly higher bioactive and proteolytic activity ($p < 0.05$). During refrigerated storage, the concentration of bioactive peptides and proteolytic activity increased in proportion to the concentration of probiotic bacteria in the food matrix (QIII > QII > QI), while to the conventional cheese (QC), the concentration remained lower values and stable over the 14 days ($p > 0.05$). Inhibition zones < 2 mm were associated with low antagonistic activity, 2-5 mm with medium antagonistic activity, and > 5 mm with high antagonistic activity. Low antimicrobial activity was observed against *S. aureus* (0.6-1.8 mm) and *L. innocua* (0.6-1.8 mm) strains, while moderate activity was observed against *Salmonella* (0.6-2.4 mm) and *E. coli* (0.6-3.0 mm). The antibacterial activity was enhanced as the concentration of *W. coagulans* increased along the storage time in both antagonism tests. The antibacterial activity of *W. coagulans* may be related to the production of lactic acid, bacteriocin and hydrogen peroxide. These results suggest that it is possible to add a spore-forming probiotic bacterium to a fresh



cheese, with adequate survival along the gastrointestinal tract and viability in the final product throughout the storage period, capable of producing functional and antibacterial compounds

References

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PP053: *Lactococcus lactis*-HSP65 induces IL-9 production, mast cell gene expression in the colon and controls the type 1 diabetes in an experimental model.

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Type 1 Diabetes (T1D) is considered an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta cells, leading to insulin secretion deficiency and chronic hyperglycemia. Alterations in the intestinal barrier, in the mucus production, and the composition of the gut microbiota are strongly associated with the development of T1D due to exacerbated immune activation, which promotes inflammation and autoimmunity. Probiotics such as the *Lactococcus lactis* have therapeutic potential by modulating the gut microbiota and immune response. Heat shock protein 65 (HSP65), plays an important anti-inflammatory role in several models of autoimmune diseases. Here, we evaluated the effects of *L. lactis* expressing HSP65 on IL-9 expression, in the mucus production and mast cell markers expression in colon of mice with T1D induced by 40 mg/kg of streptozotocin (STZ). The recombinant probiotic was cultured in M17 agar medium and 1×10^9 CFU was administered by gavage to mice daily before and during STZ induction, totaling 10 days, and every other day after the end of disease induction for an additional 10 days. We observed that *L. lactis*-HSP65 reduced the incidence of T1D and ameliorated the hyperglycemia, and wild *L. lactis* and *L. lactis* HSP65 (LL-HSP65) significantly increased mucus production in the colon of STZ-induced T1D mice. Although we found no significant differences in the gene expression of *Muc1* and *Muc2*, we observed an increasing trend for in the LL-HSP65 group. Quantitative analysis demonstrated a significant increase in mucus in the *L. lactis*- and LL-HSP65 groups when compared with groups that did not receive the recombinant probiotic. In addition, we observed a significant increase in IL-22 gene expression in both groups that received probiotics when compared with STZ group, but no statistical differences in protein production. IL-9 production was significantly higher in the *L. lactis*-HSP65 group when compared with STZ group, suggesting an important role of this cytokine in the protection against T1D onset. We also analyzed the expression of *Mmcp1* and *Mmcp4* genes, and we observed that both genes were increased in the colon of diabetic mice that received *L. lactis*-HSP65, suggesting the presence and/or activity of mast cells in the colon. In conclusion, the probiotic *L. lactis*-HSP65 protects against T1D development, increases IL-9 and mast cell gene expression in the colon of diabetic mice. These results show that *L. lactis*-HSP65 can significantly improve intestinal barrier function and be an innovative and promising approach to controlling hyperglycemia and limiting the onset of T1D.

References

OLIVEIRA, Jefferson Elias de et al. *Lactococcus lactis* expressando HSP65 protege contra o desenvolvimento de diabetes tipo 1 por meio da indução de células dendríticas tolerogênicas



via TLR2. 2024. 130 f. Tese (Doutorado)

KHARROUBI A. T. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015;6:850.

HU C, YANG J, QI Z, WU H, et al. Heat shock proteins: Biological functions, pathological roles, and therapeutic opportunities. *MedComm. China*; 2022;3:e161.

Song AA-L, In LLA, Lim SHE, Rahim RA. A review on *Lactococcus lactis*: from food to factory. *Microb Cell Fact* [Internet]. 2017;16:55. Available from: <https://doi.org/10.1186/s12934-017-0669-x>.

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PP054: *Lactococcus lactis* expressing HSP65 regulates the immune response and protects against the development of type 1 diabetes in a TLR2-dependent manner

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Heat-shock proteins (HSPs) are molecular chaperones that help maintain cellular homeostasis under stress by assisting in protein folding and degrading misfolded proteins. Heterologous HSPs, particularly HSP65, have shown potential as therapeutic tools in autoimmune diseases by modulating immune responses. When administered to the intestinal mucosa, HSP65 induces an anti-inflammatory response, reducing the severity of conditions like arthritis and atherosclerosis. Type 1 diabetes (T1D), characterized by the autoimmune destruction of pancreatic β cells and resulting hyperglycemia, remains a challenge to manage. Novel strategies that target immunological mechanisms, such as HSP-based therapies, may control autoimmune diseases and mitigate their complications, advancing treatment possibilities for these diseases, including T1D. Here, we evaluated the role of TLR2 in the protective effects of *L. lactis* expressing HSP65 in T1D experimental models (CEUA 007/2020). HSP65 expression was induced in *L. lactis* (NCD02118) by xylose in M17 culture medium. In prophylactic-therapeutic models using mice with streptozotocin (STZ)-induced or spontaneous T1D (NOD mice), *L. lactis*-HSP65 reduced hyperglycemia and disease incidence. The reduction in hyperglycemia in the group receiving *L. lactis*-HSP65 when compared to the diabetic group receiving only PBS was also observed in the glucose tolerance test (GTT). Additionally, we observed that *L. lactis*-HSP65 preserved insulin expression, reduced the degree of insulinitis and decreased serum IgG anti-HSP65 levels in the serum of mice with STZ-induced diabetes. Wild-type and recombinant *L. lactis* increased the expression of *Il22*, *Reg3g* and *Ocln* genes in the colon, whereas only *L. lactis*-HSP65 increased *ZO-1*, suggesting that both strains appear to improve intestinal barrier function. We observed that *L. lactis*-HSP65 increased the gene expression of *Ido*, *Aldh1a2*, *Pd11*, *Tgfb* and *Irf8* in the colon, which are markers of tolerogenic DCs. *L. lactis*-HSP65 increased cDC1 XCR1⁺ TLR2⁺ and PD-1⁺ Tregs in cecal lymph nodes, and TGF- β production in colon when compared with STZ+PBS group. No differences were observed in the cytokines IL-10, IFN- γ and IL-17 in the colon of mice that received probiotic. In pancreatic lymph nodes, we observed that *L. lactis*-HSP65 increased LAP⁺ T regs, in association with elevated levels of IL-10 and TGF- β in the pancreas. In TLR2-deficient mice, *L. lactis*-HSP65 did not effectively control hyperglycemia or disease incidence, correlating with reduced cDC1 XCR1⁺ and Tregs and lower IL-10 production. *In vitro*, we observed that TLR2- or IL-10-deficient bone marrow-derived dendritic cells (BMDCs) showed impaired Treg induction. These findings demonstrate that *L. lactis*-HSP65 protects against T1D via a TLR2/IL-10-dependent mechanism involving immunoregulatory Tregs and tolerogenic dendritic cells.



References

Katsarou, A., Gudbjörnsdóttir, S., Rawshani, A. et al. Type 1 diabetes mellitus. *Nat Rev Dis Primers* 3, 17016 (2017). <https://doi.org/10.1038/nrdp.2017.16>

Rajesh Rajaiah, Kamal D. Moudgil, Heat-shock proteins can promote as well as regulate autoimmunity, *Autoimmunity Reviews*, Volume 8, Issue 5, 2009, Pages 388-393, ISSN 1568-9972, <https://doi.org/10.1016/j.autrev.2008.12.004>.

Guimaraes MAF, Pinheiro-Rosa N, Oliveira RP, Aguiar SLF, Miranda MCG, Lemos L, Souza AL, Dos Reis DS, Medeiros SR, Gonçalves WA, Amaral SS, Pinho V, Miyoshi A, Azevedo VAZ, Rezende RM, Faria AMC. Hsp65-producing *Lactococcus lactis* inhibits experime

Khan, F.U.; Khongorzul, P.; Raki, A.A.; Rajasekaran, A.; Gris, D.; Amrani, A. Dendritic Cells and Their Immunotherapeutic Potential for Treating Type 1 Diabetes. *Int. J. Mol. Sci.* 2022, 23, 4885. <https://doi.org/10.3390/ijms23094885>

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PP055: Contextual effect and safety of probiotics consumption in intestinal inflammatory processes

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The intestinal mucosa is constantly exposed to various environmental antigens, originating from pathogens, or of harmless origin such as dietary components or antigens from the commensal microbiota, which affect immune homeostasis. Therefore, the immune system associated with the intestinal mucosa requires a complex cellular network of highly regulated mechanisms in order to maintain tissue balance and, at the same time, protect against the entry of pathogens. Therefore, the immune system associated with the intestinal mucosa requires a complex cellular network of highly regulated mechanisms in order to maintain tissue balance and, at the same time, protect against the entry of pathogens. Such mechanisms include specialized dendritic cells (DCs) (which express the CD103+CD11b- and CD103+CD11b+ molecules) that induce canonical responses, such as regulatory T cells (Treg) that control inflammatory responses and promote tolerance to harmless antigens, and effector cells (particularly Th17 lymphocytes) that promote tissue-specific barrier immunity. Such barrier response encompasses specialized epithelia, the production of antimicrobial molecules, antibodies, and mononuclear phagocytes. Although the intestinal mucosal immune system is constantly shaped by host genetic factors, resident microbiota, dietary habits, and exposure to environmental pathogens, the result of these interactions is usually a return to tissue homeostasis. However, disruption of the balance between tolerance and barrier immunity can lead to chronic inflammatory diseases, such as inflammatory bowel diseases (IBD). In the Mucosal Immunology Lab, we have been studying how probiotics, dietary changes, or infection episodes, shape the gut-associated mucosal immune system. We observed that, after infection clearance, *Yersinia pseudotuberculosis* (YP) causes a permanent remodeling of the immune and lymphatic systems of the gastrointestinal tract. This process, named by us “immunological scarring”, is directly related to susceptibility to experimental colitis as it compromises the migration of tolerogenic dendritic cells (DCs) to mesenteric lymph nodes. Here, we tried to reverse the immunological scarring by using two different supplements: (1) an inactivated *Saccharomyces cerevisiae* strain enriched with selenium (Se) and (2) the active yeast *S. cerevisiae* (SC) (strain UFMG A-905). Our group hypothesized that both treatments could recover the mesenteric lymphatic integrity and the migratory capacity of the CD103+ DCs, crucial for inducing tolerogenic responses in the intestine. Testing on C57BL/6 mice involved treatment 2 weeks post-infection, to reverse the immunological scar, continuing until pathogen elimination to prevent its establishment. The results obtained until now showed that post-infection treatment with the yeast SC UFMG A-905, contrary to our initial hypothesis,



aggravated chronic inflammation in previously infected animals, indicated by increased recruitment of neutrophils and Th1 cells to the mesentery and mesenteric lymph nodes (MLNs) post-infection and animal mortality. We also observed that neither treatment was sufficient to restore the integrity of the mesenteric lymphatic vessels, since the treated animals also presented reduced frequency of CD103+CD11b⁻ and CD103+CD11b⁺ DCs in the MLNs. Although these treatments have proven efficacy against intestinal inflammatory processes, we hypothesize that their beneficial effect on the mucosa is contextual and that probiotics consumption during immunological scarring may further weaken the intestinal barrier.

References

- FONSECA, D. DA et al. Microbiota-Dependent Sequelae of Acute Infection Compromise Tissue-Specific Immunity. *Cell*, v. 163, n. 2, p. 354–366, out. 2015.
- SAMANTHA et al. Evaluation of a functional craft wheat beer fermented with *saccharomyces cerevisiae* UFMG a-905 to treat salmonella typhimurium infection in mice. *Probiotics and antimicrobial proteins*, v. 15, p. 1180—1192, 2023.
- DE CAMPOS FRAGA-SILVA, T. F. et al. Selenization of *S. cerevisiae* increases its protective potential in experimental autoimmune encephalomyelitis by triggering an intestinal immunomodulatory loop. *Scientific Reports*, v. 10, p. 22190, 17 dez. 2020.
- AM;LAMMERS, S. Multiple Lactobacillus Infections Caused by Probiotics at Pediatric and Adult Academic Medical Centers. *WMJ : official publication of the State Medical Society of Wisconsin*, v. 123, n. 4, set. 2024.

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PP056: Probiotic Minas Frescal Cheese Shows Therapeutic Effects on the Attenuation of Ulcerative Colitis in a Murine Model

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Inflammatory bowel diseases (IBDs) constitute disturbances of the gastrointestinal tract that cause irreversible changes in the structure and function of tissues. Ulcerative colitis (UC), the most frequent IBD in the population, is characterized by prominent inflammation of the human colon. Functional foods containing probiotic bacteria have been studied as adjuvants to treating or preventing IBDs. Cheese is an adequate probiotic carrier with several effects on the health and presents technological advantages when compared to fermented milk. The selected probiotic strain *Lactococcus lactis* NCDO 2118 (*L. lactis* NCDO 2118) exhibits immunomodulatory effects, with promising results in the UC mouse model induced by dextran sodium sulfate (DSS). Additionally, Minas Frescal cheese is a typical Brazilian dairy food with high nutritional value, besides being a good delivery system that can be used to improve survival and enhance the therapeutic effects of probiotic bacteria in the host. Therefore, this work investigated the probiotic therapeutic effects of an experimental Minas Frescal cheese containing *L. lactis* NCDO 2118 in DSS-induced colitis in mice. Besides that, the gross composition and generation of bioactive compounds were evaluated. Adding *L. lactis* NCDO 2118 did not significantly affect ($p > 0.05$) the proximate composition and mineral content of Minas Frescal cheese, compared with conventional cheese. In addition, the generation of bioactive compounds, such as antioxidant potential (DPPH), ACE inhibitory activity (ACEI), α -amylase, and α -glucosidase on the probiotic cheese containing *L. lactis* NCDO 2118, presented increased values and were significantly different ($p < 0.05$) compared with conventional cheese. During colitis induction, mice that consumed the probiotic cheese exhibited reduced severity of colitis, with attenuated weight loss, lower disease activity index, limited shortening of the colon length, and reduced histopathological score. Moreover, probiotic cheese administration increased gene expression of tight junction proteins ZO-1, ZO-2, occludin, and claudin-1 in the colon and increased IL-10 release in the spleen and lymph nodes. Overall, Minas Frescal cheese containing the well-characterized probiotic bacteria *L. lactis* NCDO 2118 was able to alleviate the severity of DSS-induced colitis in a mice model, limiting histopathological damages, restoring intestinal barrier by increased expression of genes related to tight junction protein, and modulating the cytokine production in mice. Therefore, this study demonstrated that consumption of probiotic Minas Frescal cheese, containing *L. lactis* NCDO 2118, prevents the inflammatory process during DSS-induced colitis in mice, opening perspectives for the development of new probiotic functional foods for personalized nutrition in the context of IBD.

References

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PP057: EFFECTS OF MINAS CHEESE ENRICHED WITH WEIZMANNIA COAGULANS GBI-30 ON OXIDATIVE STRESS AND BIOCHEMICAL PARAMETERS IN A PRÉ-CLINICAL MODEL OF TYPE 2 DIABETES

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The Gut microbiota modulation through probiotic consumption offers a promising approach for treating type 2 diabetes mellitus (T2DM) by reducing factors associated with oxidative stress. Minas cheese represents a stable vehicle for the survival of cultures and the viability of probiotics. This study investigated the effects of a probiotic Minas cheese with *Weizmannia coagulans* GBI-30 on oxidative stress and biochemical parameters in rats streptozotocin-induced T2DM. Thirty-two male Wistar rats were divided into 2 groups: Control (CT, n=8) and T2DM (DM, n=24). The DM group received a high-fat diet for 4 weeks and an intraperitoneal injection of streptozotocin (35mg/kg) after the 3rd week. After 4 weeks, the DM group was divided into 3 groups (n=8/group): DM, T2DM + Minas Frescal Cheese (DMC), and T2DM + Minas Frescal Cheese with probiotics (DMPC). The DMC and DMPC group received 20g/day of cheese for 2 weeks. The probiotic cheese was added 10⁸ to 10⁹ UFC/day of *Weizmannia coagulans* GBI-30. After 6 weeks, fasting glucose was measured, and the animals were euthanized. Oxidative stress parameter was analyzed in plasma and cardiac tissue by Thiobarbituric acid reactive species) and biochemical markers in serum. (Ethical committee 6165130722). In the 4th week, the DM group showed a significant increase (369.2±55.9 vs. 93.13±10.40; p<0.0001) in fasting glycemia compared to CT, demonstrating efficacy in inducing DM2. The DMC group showed a significant reduction in fasting glycemia at week 6 compared to the DM group (342.6±15.94 vs 398.0±42.06; p=0.0028). Regarding cheese consumption, no significant difference was found. About biochemical parameters there was a significant increase in triglyceride levels in the DMC (430.2±137.9 vs. 192.6±98.08; p=0.0023) and DMPC (345.3±139.7 vs. 192.6±98.08; p=0.0459) groups compared to the DM group. A significant decrease in total cholesterol levels was observed in the DMPC group compared to the DMC group (113.0±17.71 vs. 151.0±38.32; p=0.0081), while both the DMC (151.0±38.32 vs. 66.14±10.46; p<0.0001) and DMPC (113.0±17.71 vs. 66.14±10.46; p=0.0010) groups showed an increase compared to the DM group. The DMC group showed a significant increase in calcium (15.01±2.56 vs. 11.96± 0.39; p=0.0010), magnesium (4.03±0.27 vs. 3.37±0.31; p=0.0054), phosphorus (14.33±2.47 vs. 11.53±1.76; p=0.0226), uric acid (8.11±4.62 vs. 3.33±1.37; p=0.0104), and albumin (4.14±1.00 vs. 3.13±0.15; p=0.0045) in serum compared to the DM group. There was also a significant increase in total protein in the DMC (8.30±1.83 vs. 5.64±0.48; p=0.0002) and DMPC (7.29±0.76 vs. 5.64±0.48; p=0.0156) groups compared to the DM group. Concerning plasma TBARS concentration, there was no significant difference between DMPC and the DM (p=0.9935) and DMC (p=0.6025) groups. In the heart tissue, there was a significant reduction in TBARS in the DMC group (54.88±22.52 vs. 86.94±24.67; p=0.0422) compared to the DM group, but there was no significant difference



compared to DMPC. Consumption of a probiotic Minas cheese with *Weizmannia coagulans* GBI-30 for 2 weeks in a pre-clinical model of T2DM decreased total cholesterol level. However, it did not affect fasting and postprandial glucose, and oxidative stress damage parameters.

References

- 1 Food Research International , 142, April 2021, 110208
- 2 Fermentation,10(1), 8, 2024.

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PP058: Protective Effects of Butyrate Supplementation Against Inflammation and Weight Gain in C57BL/6 Mice Fed a Cafeteria Diet

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Diets rich in sugar and fat, combined with low fiber intake, have detrimental effects on gut health. The gut microbiota ferments fibers into short-chain fatty acids (SCFAs), such as butyrate, which exhibit anti-inflammatory properties and contribute to immune homeostasis. This study investigated the protective effects of butyrate supplementation in C57BL/6 mice fed a cafeteria diet, a model mimicking Western dietary patterns. Butyrate was encapsulated using ionic gelation with HEC (hydroxyethyl cellulose) in collaboration with the Nanomaterials & Nanotoxicology research group at UNIFESP-SJC. Mice were divided into four groups: (A) control (DP) fed a standard diet, (B) cafeteria diet (DC) without supplementation, (C) cafeteria diet supplemented with butyrate (BUTHEC; 8g/kg), and (D) cafeteria diet supplemented with fiber (FIBER; 9.6g/kg). Mice were monitored for weight gain and blood glucose levels over 60 days. After treatment, tissue samples (liver, intestines, adipose tissue, and feces) were collected for analysis. Gene expression was evaluated via qPCR, focusing on intestinal and hepatic markers associated with inflammation, glucose metabolism, and gut barrier integrity. Results showed that the BUTHEC group consumed more food than the other groups (BUTHEC vs. CF: $p=0.0038$, BUTHEC vs. FIBER: $p=0.00385$) but paradoxically exhibited lower weight gain compared to the FIBER group ($p=0.0326$). Gene expression analysis revealed that butyrate supplementation increased the expression of *Alpi* (intestinal alkaline phosphatase), *Tjp1* (tight junction protein), *Ffar2*, and *Ffar3* (SCFA receptors) in the ileum compared to the cafeteria diet group. Notably, *Alpi*, induced by both butyrate and fiber, likely reduced inflammation by neutralizing bacterial endotoxins. Furthermore, butyrate-specific induction of *Tjp1* suggested improved gut barrier integrity, potentially reducing intestinal permeability and systemic inflammation. In the liver, butyrate supplementation led to a significant downregulation of CD36, a gene involved in lipid uptake and metabolism, highlighting its role in modulating hepatic lipid homeostasis. The reduced expression of CD36 likely contributed to the observed attenuation in weight gain by limiting lipid accumulation and improving metabolic efficiency. Notably, while both butyrate and fiber influenced overlapping pathways, the BUTHEC group exhibited unique and pronounced metabolic advantages. These included not only protection against excessive weight gain but also enhanced regulation of inflammatory



pathways and improved glycemic profiles, underscoring the specific benefits of butyrate beyond those provided by dietary fiber alone. These findings underscore the therapeutic potential of butyrate as a dietary supplement to counteract the deleterious effects of high-fat and high-sugar diets, particularly in the context of modern dietary patterns linked to metabolic disorders. By improving intestinal barrier function, modulating gene expression related to inflammation, and reprogramming hepatic lipid metabolism, butyrate offers a multifaceted approach to addressing obesity, insulin resistance, and other metabolic dysfunctions. While these preliminary results are compelling, further investigations are essential to validate these outcomes, including detailed histological analyses of liver and intestinal tissues. Additionally, longitudinal studies exploring the long-term impact of butyrate supplementation on metabolic health are warranted. These insights could pave the way for the development of innovative functional foods and nutraceuticals aimed at preventing and managing metabolic disorders, such as obesity, type 2 diabetes, and metabolic dysfunction-associated steatohepatitis (MASH).

References

- Magro, D. O., Rossoni, C., Saad-Hossne, R. & Santos, A. INTERACTION BETWEEN FOOD PYRAMID AND GUT MICROBIOTA. A NEW NUTRITIONAL APPROACH. *Arq Gastroenterol* 60, 132-136 (2023). <https://doi.org/10.1590/S0004-2803.202301000-15>
- Parlato, M. et al. Human ALPI deficiency causes inflammatory bowel disease and highlights a key mechanism of gut homeostasis. *EMBO Mol Med* 10 (2018). <https://doi.org/10.15252/emmm.201708483>
- Chelakkot, C., Ghim, J. & Ryu, S. H. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp Mol Med* 50, 1-9 (2018). <https://doi.org/10.1038/s12276-018-0126-x>
- Shimizu, H. et al. Dietary short-chain fatty acid intake improves the hepatic metabolic condition via FFAR3. *Sci Rep* 9, 16574 (2019). <https://doi.org/10.1038/s41598-019-53242-x>

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PP059: Characterization of a novel circular bacteriocin, streptocyclin BTW, and its diversity in the *Streptococcus* genus

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Streptococcus is a genus of gram-positive, ubiquitous bacteria that can also be found in the mouth, upper respiratory tract, and lower genital tract of humans. This group of microorganisms has been associated with severe infections and the development of resistance to antibiotics[1,2]. However, another characteristic that has been studied regarding this group is the production of bacteriocins. These compounds can be defined as ribosomally synthesized multifunctional peptides produced by prokaryotes, which can present a narrow or broad inhibitory spectrum. This class of peptides is usually hydrophobic and cationic, with the bacteriocinogenic strain presenting immunity to its own antimicrobial peptide. Bacteriocins can be divided into two main classes: (i) those with post-translational modifications and (ii) non-modified peptides[3]. Many class I bacteriocins have been described for the *Streptococcus* genus, especially lanthipeptides; however, only one circular bacteriocin was described for the group (uberolysin)[4]. The lack of characterized peptides of this group represents a promising field of knowledge to be explored. Due to this, this study has as its objective the characterization of streptocyclin, a 64-amino-acid circular bacteriocin produced by *Streptococcus devriesei* DSM 19639, as well as its diversity in the *Streptococcus* genus. The deferred antagonism assay was applied in order to understand the inhibitory spectrum of strains, which included *Enterococcus*, *Streptococcus*, *Micrococcus*, *Lactococcus*, *Listeria*, and *Clostridium* strains. Important to highlight that strain presented a pronounced activity against the *Streptococcus* genus. After, the colony mass spectrum of the strain was collected, where the mass of 6118.75 Da was observed, which matched with the predicted mass of the streptocyclin BTW. The genomic analysis revealed that the streptocyclin BTW gene cluster is composed of five genes: core peptide (*stpA*), membrane protein (*stpB*), DUF95 family protein (*stpC*), ATP-binding protein (*stpD*), and putative immunity protein (*stpE*). When compared to other characterized circular bacteriocins, its closest relatives are caledonicin (50%), amylocyclicin (43.75%), amylocyclicin CMW1 (42.19%), and enterocin NKR-5-3B (40.63%). To further understand the distribution of this bacteriocin in the *Streptococcus* genus, a sequence similarity network was used with the propeptide of streptocyclin BTW as the driver sequence. The results reveal the presence of streptocyclin-BTW-like circular bacteriocins was found only in *Streptococcus orisasini*, *Streptococcus equi*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, and *Streptococcus mitis* species. Purification of streptocyclin was done using the Jupiter Proteo semi-preparative column, running a 30-55% gradient over 5 minutes followed by a 55-100% gradient over 45 minutes, confirming its molecular mass of 6118 ± 1 Da. As far as we are concerned, this is the first report of a bacteriocin produced by



the *Streptococcus devriesei* species and the second circular bacteriocin from the *Streptococcus* genus.

References

- 1 - Gergova, R., Boyanov, V., Muhtarova, A., & Alexandrova, A. (2024). A Review of the Impact of Streptococcal Infections and Antimicrobial Resistance on Human Health. *Antibiotics* (Basel, Switzerland), 13(4), 360. <https://doi.org/10.3390/antibiotics13>
- 2 - Okahashi, N., Nakata, M., Kuwata, H., & Kawabata, S. (2022). Oral mitis group streptococci: A silent majority in our oral cavity. *Microbiology and immunology*, 66(12), 539–551. <https://doi.org/10.1111/1348-0421.13028>
- 3 - Sugrue, I., Ross, R. P., & Hill, C. (2024). Bacteriocin diversity, function, discovery and application as antimicrobials. *Nature reviews. Microbiology*, 22(9), 556–571. <https://doi.org/10.1038/s41579-024-01045-x>
- 4 - Wirawan, R. E., Swanson, K. M., Kleffmann, T., Jack, R. W., & Tagg, J. R. (2007). Uberolysin: a novel cyclic bacteriocin produced by *Streptococcus uberis*. *Microbiology* (Reading, England), 153(Pt 5), 1619–1630. <https://doi.org/10.1099/mic.0.2>

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PP060: THERMIC MODIFICATION OF PASSION FRUIT FLOUR TO PRODUCE FERMENTABLE SACCHARIDES

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The intestinal microbiota is a complex ecosystem comprising bacteria, fungi, viruses, archaea, and protozoa that metabolize available substrates through species-specific metabolic pathways. The products of these processes, including beneficial metabolites, contribute to host health, classifying the substrates as prebiotics. While many prebiotic candidates are low-molecular-weight compounds, insoluble dietary fibers indirectly modulate the microbiota by supporting fecal consistency and nutrient transport. A promising approach to enhancing prebiotic effects involves reducing the complexity of polysaccharides and lowering their degree of polymerization, thereby improving solubility and metabolic utilization by intestinal bacteria. Pectin represents nature's most structurally complex polysaccharides. When pectin is broken down (depolymerization to produce oligosaccharides), pectin-derived oligosaccharides (POS) are given rise. These oligosaccharides demonstrate enhanced solubility and metabolic potential as prebiotics. Passion fruit (*Passiflora edulis f. flavicarpa* O. Deg.) is widely cultivated in Brazil, rich in pectins and composed of soluble and insoluble fibers. The peel and albedo, byproducts of this fruit, have potential added value and are currently marketed as flour, with economic and environmental relevance. Therefore, the present study aims to evaluate the prebiotic potential of oligosaccharides obtained from modified passion fruit pectin, aiming at a sustainable formulation that uses passion fruit flour, allowing insoluble fibers to act synergistically with POS. To produce fermentable saccharides passion fruit flour is used through thermal hydrolysis. Passion fruit flour was treated at 121°C for 1 hour in the autoclave. The soluble fractions were dried using a spray dryer, while the insoluble fraction was lyophilized. The resulting samples were analyzed using high-performance size-exclusion chromatography (HPSEC) and *in vitro* colonic fermentation. Thermal treatment effectively reduced the average molecular weight of the polysaccharides, facilitating faster fermentation of the soluble fractions. Fermentation of these fractions occurred within 8 hours, demonstrating a profile comparable to the fructooligosaccharides (FOS) standard. The observed pH variation (6.85 to 5.89) and pressure changes (0 to 7.33 psi) during the fermentation of the soluble fractions closely mirrored those of FOS fermentation, where the pH ranged from 6.94 to 5.01 and pressure from 0 to 11.5 psi. These findings support the hypothesis that intestinal bacteria utilize lower molecular weight saccharides more efficiently. Further analyses, including short-chain fatty acid (SCFA) quantification and fecal microbiota composition assessment via 16S rDNA sequencing, will provide deeper insights into the fermentation dynamics, ecosystem interactions, and the role of the substrate. These data will help elucidate the prebiotic potential of modified passion fruit flour and its capacity to modulate the intestinal microbiota.



References

DE OLIVEIRA, D. P.; TODOROV, S. D.; FABI, J. P. Exploring the prebiotic potentials of hydrolyzed pectins: mechanisms of action and gut microbiota modulation. *Nutrients*, v. 16, n. 213689, 2024. DOI: <https://doi.org/10.3390/nu16213689>.

GIBSON, Y.; ROBERFROID, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*. Disponível em: <https://academic.oup.com/jn/article-abstract/125/6/1401/4730723>. Acesso em: 28 jan. 20

PEDROSA, L. F.; KOUZOUNIS, D.; SCHOLS, H.; DE VOS, P.; FABI, J. P. Assessing high-temperature and pressure extraction of bioactive water-soluble polysaccharides from passion fruit mesocarp. *Carbohydrate Polymers*, v. 335, 2024. DOI: <https://doi.org/10.1016>

SANDERS, M. E.; MERENSTEIN, D. J.; REID, G.; GIBSON, G. R.; RASTALL, R. A. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology and Hepatology*, London: Nature Publishing Group, 2019. DOI:

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PP061: Lyophilized Symbiotic Mitigates Mucositis Induced by 5-Fluorouracil

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Mucositis is an adverse effect of cancer chemotherapies using 5-fluorouracil (5-FU). It is characterized by mucosal inflammation, pain, diarrhea, and weight loss. Some studies reported promising healing effects of probiotic strains associated with prebiotics as adjuvant treatment of mucositis. The development of alternative or adjuvant treatments is needed and the use of probiotics;synbiotic as promising candidates for adjuvant treatment of mucositis recently attracted attention. This study aimed to develop a symbiotic and lyophilized product, based on milk, supplemented with WPI and FOS, fermented by strains *L. casei* BL23, *L. plantarum* B7, and *L. rhamnosus* B1, which would be able to reduce the intestinal inflammation, to control the pro-inflammatory immune response, and to decrease intestinal permeability, in a murine model of mucositis induced by 5-FU. A lyophilized synbiotic product containing skimmed milk, supplemented with whey protein isolate (WPI) and with fructooligosaccharides (FOS), and fermented by *Lacticaseibacillus casei* BL23, *Lactiplantibacillus plantarum* B7, and *Lacticaseibacillus rhamnosus* B1. Regarding the intestinal permeability, this parameter was determined in vivo by quantifying blood radioactivity after oral administration of ^{99m}Tc-DTPA. Finally, histological damages caused by 5-FU-induced mucositis were monitored. *L. casei* BL23, *L. plantarum* B7, and *L. rhamnosus* D1, submitted to lyophilization counts after rehydration of the product, reached populations greater than 2×10^9 CFU/g. The Symbiotic treatment significantly reduced ($p < 0.05$) the loss, compared to 5-FU (inflamed control group) and *L. casei* BL23, besides a slight weight loss in the Naive group without inflammation; in addition, the Symbiotic formulation showed discrete destruction and reduction of villi, moderate inflammatory infiltrate with mucosal and submucosal involvement, with moderate loss of crypts, can be observed. Finally, animals treated with matrix and Symbiotic exhibited significantly decreased ($p < 0.001$) intestinal permeability compared to the 5-FU inflamed control group. Overall, the symbiotic formulation presented anti-inflammatory potential in 5-FU-induced mucositis, reducing animal weight loss intestinal permeability, modulating genes implicated in the intestinal epithelial barrier, controlling pro-inflammatory cytokine levels, and reducing mucosal damage caused by chemotherapy. Indeed, the consumption of the symbiotic formulation caused a reduced score of inflammation in the duodenum, ileum, and colon, as well as decreased levels of pro-inflammatory cytokines IL-1 β , IL-6, IL-17, and TNF- α in the mice ileum. Therefore, the symbiotic product developed in this work thus represents a promising adjuvant treatment of mucositis. Overall, this work opens new perspectives for the development of functional symbiotic products for target populations, in the context of mucositis, based on smart selection of matrices and bacterial consortia.



References

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PP062: EVALUATION OF THE BACTERIOSTATIC ACTIVITY OF A POTENTIALLY PROBIOTIC LACTIC CULTURE AGAINST FOOD CONTAMINATION INDICATORS

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Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. These microorganisms can also contribute to the biopreservation of food, improving food safety by inhibiting spoilage and pathogenic microorganisms through the production of primary and/or secondary metabolites. The present study aimed to evaluate the minimum inhibitory activity of the potentially probiotic autochthonous *Limosilactobacillus mucosae* CNPC007 against important microbial indicators of food contamination. The inoculum with *L. mucosae* cells (I) in de Man Rogosa Sharpe broth (MRS), non-neutralized supernatant (S), or neutralized supernatant (NS), prepared with sterile NaOH (4 N) up to reach pH 7.0, were used. To determine the minimum inhibitory concentration (MIC) against the microbial indicators, the reference strains *Staphylococcus aureus* ATCC 25923 and *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* ATCC 14028, as well as a clinical isolate of *Escherichia coli*, were used. The S and NS samples were obtained after centrifugation of the culture of *L. mucosae* CNPC007 containing a population around 1.2×10^8 CFU/100 μ L, for 10 minutes at $11,000 \times g$, 3 times. The microplate microdilution method was used to determine the MIC with 100 μ L of sterile Mueller Hinton (MH) broth distributed in the wells to perform serial dilutions. Subsequently, 100 μ L of I, S and NS were added, obtaining concentrations of 50%, 25%, 12.5%, 6.25% and 3.12%. The contamination indicator suspensions were added at a concentration of 105 CFU /100 μ L to each well. As a positive control for turbidity (T), 100 μ L of the standardized suspension of the contamination indicators added to the sterile MH broth was used. As a negative control (NC), only the sterile MH broth was used. As a positive control for antimicrobial activity (PC), 100 μ L of the standardized suspension of the contamination indicators added with 100 μ L of ciprofloxacin at a concentration of 0.3 g/100 μ L were used. The microplates were incubated for 24 h at 35 ± 2 °C, and the MIC was determined by evaluating the turbidity of the well; S was able to inhibit the growth of all indicators at a concentration of 12.5%, NS did not show inhibitory activity and, due to limitations of the method, it was not possible to determine the MIC of I. According to the results, it is suggested that the antimicrobial activity of *L. mucosae* is related to the production of organic acids during the metabolization of glucose present in the MRS broth. Thus, the non-neutralized supernatant of *L. mucosae* CNPC007 strain grown in MRS broth



demonstrated bacteriostatic capacity against the microbial contamination indicators studied in vitro and may be promising as a natural preservative for the food sector.

References

Imagem marcado/desmarcado MORAES, G. M. D. de; ABREU, L. R. de; EGITO, A. S. do; SALLES, H. O.; SILVA, L. M. F. da; NERO, L. A.; SANTOS, K. M. O. dos. Functional properties of *Lactobacillus mucosae* strains isolated from Brazilian goat milk. Probiotics and GALDINO, I. K. C. P. DE O.; SILVA, M. O. M. DA; SILVA, A. P. A. DA; SANTOS, V. N.; FEITOSA, R. L. P.; FERREIRA, L. C. N.; DANTAS, G. C.; PEREIRA, E. V. DOS S.; OLIVEIRA, T. A. DE; SANTOS, K. M. O. dos; EGITO, A. S. do; BURITI, F. C. A.; CARDARELLI, H. R.

Acknowledgements:



PP063: Gut Microbiome Composition and Its Association with Dietary Factors in Crohn's Disease

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Introduction: The gut microbiota plays a crucial role in the pathogenesis and progression of Crohn's Disease (CD) by maintaining intestinal homeostasis, supporting metabolic and immune functions, and influencing overall health. An imbalance in the gut microbiota is associated with CD, as disruptions in microbial composition can be further impacted by dietary factors, highlighting the connection between nutrition and gut health.

Aim: This study aimed to evaluate the gut microbiota composition and its interactions with dietary factors to identify potential opportunities for targeted probiotic interventions in CD management.

Methods: The study included 53 participants, of whom 20 had CD in remission, 18 had active disease, and 15 were healthy controls. CD activity was evaluated by colonoscopy assessment or by nuclear magnetic resonance scan enterography. All participants aged between 18 and 60 years were assessed using 24-hour dietary recall and gut microbiome profiling through 16S rRNA gene sequencing. Patients had confirmed diagnoses of CD with ileal and/or colonic involvement. The local ethics committee approved this study.

Results: Microbial composition analyses (beta diversity) revealed significant differences between groups. At the Operational Taxonomic Unit (OTU) level, healthy microbiome differed significantly from both remission and active CD patients, with healthy controls showing a more similar microbial composition compared to remission ($p = 0.001$) and active disease ($p = 0.001$). In addition, Shannon diversity analyses demonstrated that healthy individuals exhibited significantly higher OTU diversity compared to CD patients, further highlighting the microbial imbalance associated with the disease. Among specific taxa, *Eubacterium hallii*, a key butyrate producer, was significantly reduced in patients with active CD. This microorganism is integral to intestinal metabolic balance, playing essential roles in energy homeostasis, colonic motility, immunomodulation, and inflammation suppression. Furthermore, *E. hallii* plays a critical role in bile acid and cholesterol metabolism, highlighting its importance in gut health. Disease severity was associated with changes in microbial abundance, with specific taxa colonizing the gut at higher levels as disease activity increased, including *Escherichia coli*, *Escherichia-Shigella*, and taxa from the *Enterobacteriaceae*, *Lachnospirales*, and *Bacteroides*. This growth may be linked to inflammatory processes and intestinal dysbiosis, suggesting a potentially pathogenic role. Furthermore, dietary components such as saccharin, an artificial sweetener,



were significantly associated with alterations in microbial composition. **Conclusions:** These findings underline the potential for probiotics targeting specific taxa, such as *E. hallii*, to restore microbial balance and improve clinical outcomes in CD. The study also highlights the impact of dietary components, such as saccharin, in disrupting the gut microbiome, suggesting that certain dietary factors may contribute to microbial imbalance and inflammation in CD. Future research should focus on integrating dietary patterns with probiotic development to optimize gut health and mitigate inflammation in CD patients.

References

BOLTE, L. A. et al. Long-term dietary patterns are associated with pro-inflammatory and anti-inflammatory features of the gut microbiome. *Gut*, p. gutjnl-2020-322670, 2 abr. 2021.

FAN, Y.; PEDERSEN, O. Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology*, v. 19, n. 1, p. 55–71, 4 jan. 2021.

GILL, P. A. et al. The Role of Diet and Gut Microbiota in Regulating Gastrointestinal and Inflammatory Disease. *Frontiers in Immunology*, v. 13, n. April, p. 1–22, 5 abr. 2022.

Acknowledgements:



PP064: Biopharmaceuticals of Plant Origin with Health Importance

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The study of natural products of plant origin in all areas of modern medicine, agriculture, biology and others is increasingly relevant due to the great possibilities for application. Thus, with natural products of plant origin, a number of progressive poly-pathogenic neurodegenerative diseases are successfully influenced, in which the combination of various mechanisms and risk factors causes anatomical, cellular and molecular changes, leading to disruption of higher cortical functions, memory deficits, learning disorders, behavior and functions and a total collapse of intelligence and mental activity. Amyloid-activated microglial cells release proinflammatory cytokines. The study of the sequence of amino acids in A β 42-peptides allows the specification of their precursor. Its abnormal degradation leads to the formation of A β 42-peptides, which are involved in amyloidogenesis and neurotoxic adhesive amyloid peptides: 90% A β 40-peptides and 10% A β 42- involved in amyloidogenesis. It has been established that a dynamic equilibrium exists in the body between the formation of reactive oxygen species and the function of protective antioxidant systems (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase). Bulgarian pharmacologist D. Paskov studies the alkaloid Galantamine is most widely distributed in representatives of the family Amaryllidaceae: Caucasian snowdrop (*Galanthus woronowii* Losinsk), snowdrop (*Galanthus nivalis* L., *Galanthus elwesii* Hook. Fil. marsh snowdrop (*Leucojum aestivum* L.) - Pharmacological studies of Galantamine prove: nervous system: 1) reversibly inhibits acetylcholinesterase, which leads to the accumulation of acetylcholine and to indirect cholinomimetic effects, 2) allosterically potentiates α 7-subtype nicotinic acetylcholine receptors, thereby enhancing dopamine and γ -aminobutyric acid neurotransmission, 3) improves learning and memory processes. Unsaturated higher fatty acids with 20 or more carbon atoms, three or more double bonds are essential components of cell membranes, can modulate physiological processes including membrane transport, receptor function and enzyme activities, and have been shown to have marked effects on various immunological and haemostatic parameters. In Alzheimer's disease, oxidative stress occurs as a result of a disturbance in the balance between endogenous or exogenous overproduction of reactive oxygen species and a decrease in antioxidant defense mechanisms. Parkinson's disease (PD) is considered the second most common neurodegenerative disorder that affects approximately 1% of the world population older than 65 years, representing up to two-thirds of all patients with movement disorders throughout the world. PD has become increasingly



more common with advances in age, reaching the proportions of 2.6% of the population over 85 years old .In addition, some data suggest that by 2020, motor development disorders will be developed worldwide as secondary to PD . During the pathogenesis of PD, the production of oxygen-reactive species damages the substantia nigra through lipid peroxidation, protein oxidation, and DNA oxidation. The use of various substances of plant origin, such as galantamine from snowdrop, omega unsaturated fatty acids, antioxidants and other substances leads to the creation of biopharmaceutical products and food supplements, which significantly increase the quality of healthcare.

References

Food Additives Unit Chemical Safety and Toxicology Division, Food additives legislation Guidance notes, London WC2 6NH, March, 2002

Virtanen JK et al.,Fish consumption and risk ofsubclinical brain abnormalities on MRI in older adults, *Neurology*, 2008; 71: 439–446

Acknowledgements:



PP065: Plant Extracts with Antiviral Activity as Food Supplement and Alternative to Monotherapy for Viral Infections

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Viruses affect seriously humans, animals, plants and other eukaryotic organisms. They are pathogenic organisms that pose a threat to the economy on a national and global scale, affecting not only people, significantly and seriously reducing their working capacity for a significant period of time, but also to the global health of ecosystems. They are highly variable, cause epidemics and change rapidly when applied therapy and prophylaxis. Many of them are the cause of millions of deaths in the world. Currently, there are only a few preferred and proven antiviral drugs, which unfortunately are effective only against a limited set of viruses. Research on natural products can be an effective strategy for the development of new potent antiviral drugs. In this regard, plant extracts represent a significant natural resource for use against various viral infections. Viral infections represent a major challenge in medicine due to various factors such as viral mutations, new viral strains and variants, toxic effects of viral infection, severity of disease, intracellular cytopathic effects and limited availability of effective antiviral drugs. Despite the progress in immuno-prophylaxis and specific antiviral therapy, there is a need to develop new and more effective antiviral compounds and alternative highly effective therapeutic regimens. On the other hand, plants produce natural secondary metabolites (as natural defense mechanisms against various environmental pests) that show antiviral activity. These secondary metabolites include compounds of basic chemical compounds such as alkaloids, flavonoids, polyphenols, carbohydrates and essential oils. Various chemical analytical methods such as HPLC, GC-MS and NMR spectroscopy are used to identify and characterize these bioactive compounds. Flavonoids, terpenoids, lignans, sulfides, polyphenols, coumarins, and saponins are among the groups of bioactive compounds found in plants that have shown antiviral activity against viruses such as herpes, coronaviruses, HIV, influenza, hepatitis, and others. The use of different solvents and extraction techniques allows the extraction and fractionation of different complexes of active compounds with different effects, including antiviral activity. Screening of plant extracts and isolation of active compounds allows scientists to identify potential new antiviral drugs. In vitro and in vivo studies have shown significant antiviral activity of plant extracts and their bioactive compounds. The use of advanced analytical techniques helps in the identification and characterization of bioactive compounds that target different stages of the viral life cycle. Many plant extracts and compounds with antiviral activity against specific viruses, human, animal, plant, and others, have been reported in scientific publications. The present review highlights the ongoing research on natural sources, especially plants, for the discovery of new and effective antiviral compounds, their safety, drug interactions, and combination therapies. The



extraction of specific combinations of substances and complexes with antiviral activity by their extraction with different solvents represents an alternative for combating viral infections. Due to their very low or no toxicity, they can be included in food supplements to improve human health.

References

Petrov N., Stoyanova M., Gaur R.; 2021. Chapter 19 “ Ecological methods to control viral damages in tomatoes” In Plant Virus-Host Interaction. Molecular Approaches and Viral Evolution, Second ed., © 2021 Elsevier Inc, ISBN 978-0-12-821

Petrov N., Stoyanova M., Gaur R.; 2024. Chapter XIX Viruses as stress factor and their management in vegetable crops In Molecular Dynamics of Plant Stress and its Management. ISBN : 978-981-97-1698-2,605632 Springer, p. 331-350

Todorov, S., Alves, V., Popov, I., Weeks, R., Pinto, U., Petrov, N., Ivanova, I., Chikindas, M. 2024. Antimicrobial compounds in wine. Probiotics and Antimicrobial Proteins, 16:763–783

Petrov, N. Stoyanova, M., Valkova, M. 2016. Antiviral activity of plant extract from *Tanacetum vulgare* against Cucumber Mosaic Virus and Potato Virus Y. J. BioSci. Biotechnol, 5(2): 189-19, ISSN 1314-6246

Acknowledgements:



PP067: Effects of Synbiotic, Protein Supplementation Associated to Resistance Training on Inflammation, Oxidative Stress, and Muscle Strength in Older Adults with Type 2 Diabetes Mellitus: A Triple-Blinded Randomized Controlled Trial

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Background & aims: The accelerated ageing of the population represents a significant achievement of contemporary society; however, it also poses a series of challenges, particularly for developing countries, including the rise in non-communicable diseases, which can significantly increase the burden on healthcare and care services. Brazil ranks sixth globally in the number of individuals with type 2 diabetes mellitus (T2DM), with projections estimating 23.2 million cases by 2045. This places Brazil among the top ten countries with the highest prevalence of T2DM. There is an increased risk of sarcopenia with aging and T2DM, highlighting the involvement of diabetes in the pathophysiology of the musculoskeletal diseases. In addition to a low protein intake and sedentary habits, recent findings indicate that microbial dysbiosis contributes to systemic oxidative stress and the inflammaging pattern, already observed in aging process. Changes in the lifestyle of individuals with T2DM are crucial along with pharmacological interventions to improve the overall health status, since these modifications may also regulate the redox and inflammatory scenario.

Objective: This study investigated the combined effects of Protein supplementation, Synbiotic+Protein and Resistance Training (RT) on physical performance, body composition, glycemic, inflammatory and oxidative stress parameters in older adults with T2DM.



Methods: Randomized, triple-blinded clinical trial involving men aged over 65 with T2DM submitted to 12-week muscle-strength exercise protocol involving twice-weekly supervised sessions lasting 45-60 minutes. Participants (n=51) were allocated in three experimental groups: Control (only RT), Protein (20g of whey protein after RT) and Synbiotic+Protein (commercial capsules containing 10×10^{10} UFC/ml of *Lactobacillus acidophilus* NCFM, *Lactocaseibacillus paracasei* Lpc-37, *Bifidobacterium lactis* BI-07, *Bifidobacterium lactis* BI-04 and microcrystalline cellulose plus 20g of whey protein.

Results: Participants showed homogeneity in bio-sociodemographic characteristics, although the SP group had lower alcohol consumption and Protein group reported higher use of sulfonylurea medication, potentially explaining the higher fat mass, body mass index and body fat percentage. All groups showed improved physical performance, and better redox balance after interventions, as indicated by higher uric acid levels (an antioxidant) and lower pro-oxidant iron concentrations in plasma. Although all groups showed higher levels of muscle injury markers (lactate dehydrogenase and creatine kinase activities), only the Protein group demonstrated strength gains, reduced insulin resistance, and oxidative insults from heme-iron species. Pro-inflammatory cytokines IFN- γ and IL-6 were also increased in the Protein group, possibly reflecting their role as mediators of integrative energy metabolism responses. The Synbiotic+Protein group exhibited reduced IFN- γ and increased Heme-Iron and CK concentrations.

Conclusions: Resistance Training combined with Protein supplementation promoted strength gain and improved physical performance improvement some markers for inflammation and oxidative stress while the type and dosage used of Synbiotic combined with Protein and Resistance Training did not bring additional advantages.

References

- Miller EG, Nowson CA, Dunstan DW, Kerr DA, Menzies D, Daly RM. Effects of whey protein plus vitamin D supplementation combined with progressive resistance training on glycaemic control, body composition, muscle function and cardiometabolic risk factors in
Tamayo M, Olivares M, Ruas-Madiedo P, Margolles A, Espín JC, Medina I, Moreno-Arribas MV, Canals S, Mirasso CR, Ortín S, Beltrán-Sánchez H, Palloni A, Tomás-Barberán FA, Sanz Y. How Diet and Lifestyle Can Fine-Tune Gut M
Qaisar R, Burki A, Karim A, Iqbal MS, Ahmad F. Probiotics Supplements Improve the Sarcopenia-Related Quality of Life in Older Adults with Age-Related Muscle Decline. *Calcif Tissue Int.* 2024 Jun;114(6):583-591. doi: 10.1007/s00223-024-01211-6. Epub 2024 Ap
Wu J, Yang K, Fan H, Wei M, Xiong Q. Targeting the gut microbiota and its metabolites for type 2 diabetes mellitus. *Front Endocrinol (Lausanne)* 2023;14. <https://doi.org/10.3389/fendo.2023.1114424>.

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PP068: Probiotics and institutionalized older adults a pilot triple blinded randomized clinical trial

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Introduction: Due to social changes and specific services and care of elderly people, Long Stay Institution for Elderly (LSIE) emerged. These institutions should offer an environment with activities that stimulate cognition, physical capacity and a healthy lifestyle, working to maintain autonomy and delaying functional losses. During aging, changes in characteristics are observed with functional decline in different organs and systems. This deterioration is the fundamental risk factor for diseases that may be associated with aging. The muscular system suffers from the aging process resulting in sarcopenia. Furthermore, cognitive capacity may be altered, with difficulties with memory and other cognitive skills, and the presence of depression. The intestinal microbiome has been proposed as a possible determinant of healthy aging as it may influence the intestinal permeability. It may contribute positively to preventing bone and cognitive decline. In this sense, probiotic supplementation aimed at reducing intestinal dysbiosis favors to the prevention and treatment of conditions associated with aging, such as cardiovascular and neurological diseases.

Objective: This study aimed to evaluate the effect of probiotic consumption for 12 weeks on clinical and anthropometric characteristics, handgrip strength, and cognitive ability in institutionalized older adults.

Method: This is a pilot prospective randomized triple-blind study. We included 20 participants of both sexes residing at a private Long Stay Institution for Elderly in the city of São Paulo, SP, Brazil). Participants were randomly assigned to a control group (placebo) and an experimental group that was supplemented with commercial probiotics containing 1 billion of *Bifidobacterium lactis* HN019[®] and 1 billion of *Lactobacillus acidophilus* NCFM[®] for 12 weeks. Two participants in the control group were excluded due to the need for hospitalization. All participants were evaluated pre- and post-supplementation. A sociodemographic questionnaire, clinical characteristics, anthropometric measurements, handgrip strength (Jamar[®] hand dynamometer), and cognitive capacity were assessed using the Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment-Basic (MoCA-B)



questionnaire. To evaluate the effects of probiotic supplementation, the delta variation of each variable (post-pre) was calculated, presented by median, and the comparative test was performed.

Results: The sample characteristics data showed that the groups were homogeneous before the supplementation. Although some positive changes, mainly in relation to mood and social interaction, have been reported by caregivers, there was no statistical difference regarding clinical characteristics, anthropometric measurements, handgrip strength ($p=0.40$), and cognitive capacity by MMSE ($p=0.73$) and MoCA-B ($p=0.89$) between groups and before and after the supplementation.

Final considerations: Probiotic supplementation did not show any influence on the parameters evaluated. However, these findings should be further investigated with studies that correct these described limitations so that the data can be extrapolated to institutionalized elderly population.

References

- Hutchinson AN, Bergh C, Kruger K, Süsserová M, Allen J, Améen S, Tingö L. The Effect of Probiotics on Health Outcomes in the Elderly: A Systematic Review of Randomized, Placebo-Controlled Studies. *Microorganisms*. 2021 Jun 21;9(6):1344.
- van den Nieuwboer, M., Klomp-Hogeterp, A., Verdoorn, S., Metsemakers-Brameijer, L., Vriend, T.M., Claassen, E., & Larsen, O.F.A. (2015). Improving the bowel habits of elderly residents in a nursing home using probiotic fermented milk. *Beneficial Micro*
- Neeraja Recharla, Jihee Choi, Pradeep Puligundla, Seon-Joo Park, Hae-Jeung Lee, Impact of probiotics on cognition and constipation in the elderly: A meta-analysis, *Heliyon*, Volume 9, Issue 7, 2023, e18306, ISSN 2405-8440, <https://doi.org/10.1016/j.heliyon>
- Shokri-Mashhadi N, Navab F, Ansari S, Rouhani MH, Hajhashemy Z, Saraf-Bank S. A meta-analysis of the effect of probiotic administration on age-related sarcopenia. *Food Sci Nutr*. 2023 Aug 9;11(9):4975-4987. doi: 10.1002/fsn3.3515. PMID: 37701185; PMCID: PM

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PP069: Protective Effect of *Weissella paramesenteroides* WpK4 on Murine Intestinal Mucositis: The Role of Tryptophan Metabolism

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The intestinal microbiota plays a crucial role in host homeostasis, with the balance between its components defining eubiosis, a state associated with host health. Dysbiosis, on the other hand, results from the loss of this balance and may be implicated in the development of various pathological conditions. Intestinal mucositis induced by chemotherapeutic agents, such as 5-fluorouracil (5-FU), is an example of an inflammatory condition in which dysbiosis can serve as an important biomarker. In this context, probiotics have been investigated as a therapeutic approach to restore eubiosis and mitigate the adverse effects of dysbiosis. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host, and evidence suggests they may be effective in controlling dysbiosis associated with inflammatory conditions. The bacterial strain *Weissella paramesenteroides* WpK4, isolated from the nasopharynx of weaned pigs, has shown probiotic potential, exhibiting beneficial effects in experimental models of infection and inflammation. A distinctive feature of this strain is the presence of the tryptophan operon in its genome, enabling it to synthesize tryptophan (TRP), an essential amino acid involved in critical biological processes, such as modulation of inflammatory responses and cellular signaling. Therefore, this study aimed to investigate the impact of tryptophan metabolism on the protection conferred by *W. paramesenteroides* WpK4 in a murine model of 5-FU-induced intestinal mucositis. Metabolic profiling of *W. paramesenteroides* WpK4 revealed the production of TRP, tryptamine (TAM), indole-3-acetic acid (IAA), and indole aldehyde (IAld), compounds known for their bioactive properties related to tryptophan metabolism. In the 5-FU-induced mucositis model, oral administration of *W. paramesenteroides* WpK4 resulted in 100% survival of the animals, while the control group, treated with 0.9% saline solution, showed a survival rate of 60%. Moreover, animals treated with *W. paramesenteroides* WpK4 exhibited lower morbidity and preservation of the intestinal microbiota composition, characteristics suggesting favorable modulation of the inflammatory response. Molecular analysis also indicated an increase in the expression of genes related to tryptophan metabolism in the host, suggesting the activation of the AhR (aryl hydrocarbon receptor) pathway, which may be involved in the observed protection. These results indicate that *W. paramesenteroides* WpK4 could represent a promising therapeutic strategy for the treatment of chemotherapeutic-induced intestinal mucositis, with its tryptophan metabolism possibly mediating the observed protective effects. The modulation of the intestinal microbiota and activation of tryptophan signaling pathways are mechanisms that warrant further investigation for the development of probiotic-based therapies in inflammatory contexts.



References

ALVIM, L. B. et al. *Weissella paramesenteroides* WpK4 reduces gene expression of intestinal cytokines, and hepatic and splenic injuries in a murine model of typhoid fever. *Beneficial microbes*, v. 7, n. 1, p. 61-73, 2016.

Sandes S, Figueiredo N, Pedroso S et al (2020) *Weissella paramesenteroides* WpK4 plays an immunobiotic role in the gut-brain axis, reducing gut permeability, anxiety-like and depressive-like behaviors in murine models of colitis and chronic stress. *Food Re*

Rodrigues EP, Soares CDP, Galvão PG, et al (2016) Identificação de genes envolvidos na biossíntese do ácido indol-3-acético pela cepa *Gluconacetobacter diazotrophicus* PAL5 usando mutagênese de transposon. *Fr*

Giulietti A, Overbergh L, Valckx D et al (2001) Uma visão geral da PCR quantitativa em tempo real: aplicações para quantificar a expressão do gene da citocina. *Métodos* 25:386–401. <https://doi.org/10.1006/meth.2001>.

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PP070: Biocontrol potential of *Lactiplantibacillus plantarum* 9A3 isolated from an innovative alheira: characterisation of its bacteriocin(s)

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Food safety is a global concern, particularly due to the presence of pathogenic microorganisms (European Food Safety Authority and European Centre for Disease Prevention and Control, 2024). The growing demand for natural alternatives to synthetic chemical preservatives has driven research into biocontrol strategies, such as the use of lactic acid bacteria (LAB) and their antimicrobial metabolites, including bacteriocins. These antimicrobial peptides can inhibit foodborne pathogens without compromising the quality of the food product (Azevedo, I., Barbosa, J., Albano, H., Nogueira, T., & Teixeira, P., 2024).

Alheira, a traditional Portuguese sausage-like product, has evolved through innovation, including alternative formulations to meet consumer preferences for healthier, tastier, and higher-quality products. The presence of LAB, such as *Lactiplantibacillus plantarum*, in this product can help inhibit undesirable microorganisms, thereby improving food safety. However, the effectiveness of biocontrol using native strains depends on a detailed characterization of the strain and its bacteriocins, assessing factors such as the spectrum of activity, stability, and mode of action.

This study aimed to evaluate the biocontrol potential of the *L. plantarum* 9A3 strain, isolated from an innovative vegetable- and mushroom-based alheira, by characterizing its bacteriocins. More specifically, the study examined:

- i) The maximum bacteriocin production (Arbitrary Units (AU)/mL) during *L. plantarum* growth,
- ii) The bacteriocins' mode of action and antimicrobial activity spectrum,
- iii) The effects of enzymes, surfactants/detergents, pH, and temperature on bacteriocin activity,
- iv) Bacteriocin adsorption and molecular size.



Additionally, Whole Genome Sequencing (WGS) was performed to identify genes related to bacteriocin production.

Genes encoding plantaricin E, plantaricin F, pediocin, and leucocin A were identified in *L. plantarum* 9A3. The produced bacteriocins exhibited a broad spectrum of activity against several strains of *Listeria monocytogenes*, *Clostridium sporogenes*, and *Clostridium perfringens*, with an estimated molecular size ranging from 37 to 52 kDa. The bacteriocins remained stable under various conditions, including temperatures from 4 to 100 °C, pH levels from 2 to 8, and exposure to surfactants and detergents (Tween 20 and 80, SDS, EDTA at 0.1, 2, and 5 mM, urea, and sodium deoxycholate) as well as enzymes (papain and catalase). The highest activity (AU/mL = 12,800) against four *L. monocytogenes* strains was observed between 21 and 36 hours of *L. plantarum* 9A3 growth, indicating a bacteriostatic mode of action.

The studied strain appears to be a strong candidate for potential application as a protective culture in the food industry. Understanding the optimal conditions for bacteriocin production is essential to maximizing its applicability.

References

European Food Safety Authority, & European Centre for Disease Prevention and Control. (2024). The European Union One Health 2023 Zoonoses report. *EFSA Journal*, 22(12), Article e9106. <https://doi.org/10.2903/j.efsa.2024.9106>

Azevedo, I., Barbosa, J., Albano, H., Nogueira, T., & Teixeira, P. (2024). Lactic acid bacteria isolated from traditional and innovative alheiras as potential biocontrol agents. *Food Microbiology*, 119, 104450. <https://doi.org/10.1016/j.fm.2023.104450>

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PP071: Influence of the persistence of *Listeria monocytogenes* on the susceptibility to bacteriocins

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Listeria monocytogenes is a widespread microorganism that causes listeriosis, a serious disease in humans resulting from the consumption of contaminated food. While it can affect healthy individuals, listeriosis primarily impacts the elderly, immunocompromised individuals, pregnant women, and newborns, with a high case fatality rate (20%–30%) (Magalhães et al., 2023). Effective elimination of this pathogen is challenging due to its ability to form biofilms and resist conventional sanitisation measures. Within the food processing environment, *L. monocytogenes* strains can be classified as persistent (colonising industrial surfaces for long periods of time, even after successive cleaning and disinfection procedures) or non-persistent (detected sporadically and tending to be more easily eliminated) (Ferreira et. a al., 2014). The presence of persistent strains in the food industry represents an increased risk as they can act as continuous reservoirs of contamination, making it essential to develop more effective control strategies.

A promising approach to controlling *L. monocytogenes* is the use of bacteriocins, antimicrobial peptides produced by some microorganisms, such as lactic acid bacteria (LAB). Despite the proven efficacy of some bacteriocins against *L. monocytogenes*, it is still unclear whether the persistence of certain strains influences their sensitivity to these antimicrobial substances.

Therefore, this study aims to evaluate the action of different bacteriocins against persistent and non-persistent strains of *L. monocytogenes*, to understand whether there is a relationship between persistence capacity and resistance to these substances.

Antimicrobial activity (expressed as arbitrary units (AU) per mL) of semi-purified bacteriocins of six LAB strains (*Lactiplantibacillus plantarum* R23; *Pediococcus pentosaceus* K34; *P. pentosaceus* SB83; *P. pentosaceus* DT016; *Pediococcus acidilactici* HA-6111-2 and *Leuconostoc lactis* RK18) against 32 *L. monocytogenes* strains from fermented meat products (15 non-persistent and 17 persistent) was determined. With the exception of *L. lactis* RK18 (with inhibitions between 200 and 6400 AU/mL), all bacteriocins produced by the remaining LAB showed excellent anti-listerial activity (between 3200 and 12800 AU/mL). However, there was no evidence of a clear relationship between the persistence of *L. monocytogenes* and its susceptibility to the bacteriocins tested. Although some strains showed distinct resistance profiles, the data obtained do not allow the conclusion that the ability to persist is directly related to the greater or lesser efficacy of the bacteriocins.



Further research is needed to clarify the relationship explored in this preliminary study. Studies with a larger number of strains and different bacteriocins or even evaluating the efficacy of bacteriocins against *L. monocytogenes* strains subjected to stress conditions simulating industrial environments could contribute to a more in-depth understanding.

References

Ferreira, V., Wiedmann, M., Teixeira, P., & Stasiewicz, M. J. (2014). *Listeria monocytogenes* persistence in food-associated environments: Epidemiology, strain characteristics, and implications for public health. *Journal of Food Protection*, 77(1), 150&

Magalhães, R., Mena, C., Ferreira, V., ... Gibbs, P., & Teixeira, P. (2023). *Listeria monocytogenes*. In *Encyclopedia of Food Safety* (2nd ed., Vols. 1–4, pp. V2-164–V2-178). Elsevier.

Acknowledgements: This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through the project GenoPhenoTraits4Persistence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021). To the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to the CISAS, UIDB/05937/2020 (DOI: 10.54499/UIDB/05937/2020) and UIDP/05937/2020 (DOI: 10.54499/UIDP/05937/2020).



PP072: SUPPRESSION OF ABERRANT CRYPTS IN BALB/C MICE BY *Bifidobacterium animalis* ssp. *lactis* INL1

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Colorectal cancer (CRC) is considered the third highest mortality worldwide and has low remission rates. Numerous research groups have worked to discover prevention agents, or alternatives to conventional drug therapies, for intestinal syndromes, such as inflammatory bowel disease and CRC. Some experiments have demonstrated the antitumor effect of specific strains, including bifidobacteria. The mechanism of action attributed to these probiotics is not yet clear, however, their antioxidant and anti-inflammatory potential are some of the proposed mechanisms. *B. animalis* ssp. *lactis* INL1 (BAINL1) appears to have antioxidant and anti-inflammatory capacity. This research evaluated whether BAINL1 had potential antitumor activity by studying the suppression of aberrant crypts in BALB/C mice with induced colorectal cancer. In vitro antioxidant activity (DPPH and ABTS) and antitumor activity in an animal model were evaluated by counting aberrant crypts (AC) and aberrant crypt foci (ACF), in addition to analyzing enzymes involved in oxidative stress and markers of tissue inflammation (IL-10 and TNF α). The results demonstrated that exposure to BAINL1 promoted a 44.44% reduction in the number of total aberrant crypts in the diseased group, in addition to increasing catalase activity (145.20%) and the quantification of TNF α (266.31%). In vitro tests showed that the bacteria under test had antioxidant activity (79.18% by DPPH and 17.02% by ABTS). As demonstrated, BAINL1 had antioxidant activity in vitro, corroborating the results of other studies. In the animal model, Catalase activity was elevated in the presence of the probiotic, which may have contributed to a reduction in oxidative stress. BAINL1 alone was not able to modulate inflammatory mediators under study (TNF α and IL-10). In the complex context of interactions between the probiotic, endothelial cells and the adjunct immune system and commensal bacteria, it appears that interesting events can be favored by it, such as an increase in the release and IgA activity. Studies involving the dosage of this immunoglobulin, as well as IL-4, IL-6, IL-8, IL-12, among others, would be interesting in order to outline a profile of which modulators would be important in the context of probiotic exposure in the CRC induction model used. The effects of increasing this mediator are numerous and antagonistic. It is believed that this modulation can contribute to both worsening and improving the disease. Although this cytokine is primarily a pro-inflammatory mediator, in the complex context of interactions between the probiotic, endothelial cells and the adjunct immune system and commensal bacteria, it appears that interesting events can be favored by it, such as an increase in the release and furthermore, considering the various stages of CRC development. Regarding the direct microscopy stage, the results indicate that BAINL1 was able to partially suppress the appearance of aberrant figures in absolute counts, in addition to reducing the appearance of aberrant crypt foci and also improving the multiplicity profile of the number of ACF, when



administered concomitantly with the carcinogen. These results point to possible antitumor activity. This study presents support that signals the possible antitumor activity of BAINL1 on CCR induced in an animal model, for oral exposure.

References

- OH, N. S.; JOUNG, J. Y.; LEE, J. Y.; KIM, Y. Probiotic and anti-inflammatory potential of *Lactobacillus rhamnosus* 4B15 and *Lactobacillus gasseri* 4M13 isolated from infant feces. *PLoS ONE*, v. 13, n. 2, 2018.
- HILL, C.; GUARNER, F.; REID, G.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.; FLINT, H. J.; SALMINEN, S.; CALDER, P. C.; SANDERS, M. E. Expert consensus document: The International Scientific Association for Probiotics and Prebio
- DI GIOIA, D.; ALOISIO, I.; MAZZOLA, G.; BIAVATI, B. Bifidobacteria: Their impact on gut microbiota composition and their applications as probiotics in infants. *Applied Microbiology and Biotechnology*, v. 98, n. 2, p. 563–577, 2014.
- CENTANNI, M.; TURRONI, S.; RAMPELLI, S.; BIAGI, E.; QUERCIA, S.; CONSOLANDI, C.; SEVERGNINI, M.; BRIGIDI, P.; CANDELA, M. *Bifidobacterium animalis* subsp. *lactis* Bi07 modulates the tumor necrosis factor alpha-dependent imbalances of the enterocyte-associat

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PP073: DEVELOPMENT OF LACTOSE-FREE PROBIOTIC YOGURT THROUGH FERMENTATION AND ADDITION OF LACTASE IN PARTNERSHIP WITH A FAMILY FARMING COOPERATIVE IN WEST PARANÁ FOR SCHOOLCHILDREN

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Intolerance is characterized by the body's inability to hydrolyze lactose from milk and its derivatives due to the absence or reduced amount of lactase enzyme in the intestine, which can lead to diarrhea, excessive gas production, pain due to abdominal distension, flatulence, nausea and vomiting. This problem can affect different age groups and is especially complicated in children. The objective of this work was to develop a lactose-free probiotic natural yogurt, through conventional fermentation and addition of lactase, aimed at children attending public schools in a municipality in the west of Paraná, a project that is part of a partnership between UNIOESTE and a cooperative of family farmers in the region (COOPLAF). The yogurt was made using whole milk, two probiotic commercial lactic acid cultures (*L.bulgaricus* with *S.thermophilus* and *L.acidophilus*) and acid lactase, with 13% sucrose added and without added starches, colorings or preservatives. From these ingredients, three treatments were planned with addition of the enzyme at different times of manufacture: (T1) addition of lactase at 42°C with incubation for 4h followed by addition of lactic acid cultures with incubation for 4h and cooling at 10°C; (T2) addition of lactase together with the lactic acid cultures at 42°C for 4h and subsequent cooling to 10°C; (T3) addition of lactic acid cultures and incubation at 42°C followed by cooling at 10°C for 4h and addition of lactase with maintenance of the enzyme for 20h at the same refrigeration temperature (04 repetitions). The control yogurt was added from the same lactic acid cultures without the addition of lactase, incubated at 42°C for 4 hours and then refrigerated at 10°C. The products obtained were subjected to compositional and physical-chemical analyses, according to methodologies described in Brazilian Legislation. Compositional analyzes of moisture, ESD, total fat, total proteins, total carbohydrates, lactose and the physical-chemical properties of titratable acidity, pH and viscosity were carried out. The analyzes were carried out in duplicates and compared to Brazilian Legislation standards for fermented milks. The lactose content was determined using an enzymatic spectrophotometric method. The results obtained were submitted to ANOVA and when a significant difference was detected at a 5% probability level, the Tukey test was applied. Treatment 1 was the one that showed the best results, achieving total lactose hydrolysis, with an average composition of 80.95% moisture (± 4.39); 19.05% ESD (± 4.39); 3% total fat (± 0.31); 3.15% total proteins (± 0.25), 12.99% total carbohydrates in sucrose (± 5.31) and 0% lactose and average physicochemical properties of 0.67% lactic acid as acidity titratable (± 0.06); 4.40 pH (± 0.15) and 387.5cP viscosity (± 21.65), all complying with legislation. Under the conditions studied in this work, it was concluded that it was possible to produce a lactose-free full-fat natural probiotic yogurt that could contribute positively to the human intestinal



health. The aim is to carry out a product sensory evaluation among schoolchildren, using the same formulation with the suppression of the addition of sucrose, looking for a natural ingredient as a sweetener, which will make the product even healthier for the target audience.

References

- Alard, J., Peucelle, V., Boutillier, D., Breton, J., Kuille, S., Pot, B., Holowacz, S., Grangette, C., 2018. New probiotic strains for inflammatory bowel disease management identified by combining in vitro and in vivo approaches. *Beneficial Microbes*, 9: 3
- George Kerry, R., Patra, J.K., Gouda, S., Park, Y., Shin, H.S. and Das, G., 2018. Benefaction of probiotics for human health: A review. *Journal of Food and Drug Analysis*. <https://doi.org/10.1016/j.jfda.2018.01.002>
- Guimarães, J.T., Balthazar, C.F., Silva, R., Rocha, R.S., Graça, J.S., Esmerino, E.A., Silva M.C., Sant'Ana, A.S., Duarte, M.C.K.H., Freitas, M.Q., Cruz, A.G., 2020. Impact of probiotics and prebiotics on food texture. *Current Opinion*
- Roobab, U., Batool, Z., Manzoor, M.F., Shabbir, M.A., Khan, M.R., Aadil, R.M., 2020. Sources, formulations, advanced delivery and health benefits of probiotics. *Current Opinion in Food Science* 32, 17-28.

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PP074: Artisanal cheeses safety is associated with the native microbiota

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Canastra Cheese is one of seven acknowledged artisanal cheese-producing regions in Minas Gerais state, and it was recognized as Brazilian Intangible Cultural Heritage. The production of Serra da Canastra cheese uses a fermentation starter culture, popularly known as "*pingo*", which consists of a portion of the whey recovered from the previous day's production. Studies on this endogenous starter composition are especially focused on lactic acid bacteria (LAB), using traditional microbiological analysis methods. In this study we aimed to isolate a broad range of bacteria present in this traditional starter culture (*pingo*), and to characterize representative strains using traditional culture-based and genomic-based methods. We evaluated and isolated bacterial strains from *pingo* samples obtained from 10 different cheese producers using various culture media. Bacterial isolates were initially screened based on colony morphology and Gram staining. Subsequently, a collection of 113 isolates was screened by sequencing the 16S rDNA gene, to select representative isolates for full genome sequencing. These selected isolates were also characterized for biogenic amine production, hemolytic activity, gelatinase activity, bacteriocin production, mucin degradation and antimicrobial susceptibility. The results revealed a diverse microbial community dominated by LAB such as *Enterococcus faecalis*, *Streptococcus* spp., and *Leuconostoc* spp., alongside nonstarter culture, including *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, and *Rothia kristinae*. The complete genome of 11 strains was sequenced. Pangenome analysis together with ANI demonstrated similarities between the isolated LAB and others deposited in GenBank and also related to cheese production, such as *Lactococcus lactis* and *Streptococcus* sp. However, *Leuconostoc* sp., even though it is associated with cheese production, is still poorly characterized, which makes it difficult to compare to the point of knowing whether or not it is a new species. Hemolytic activity was observed in all LAB isolates, raising concerns about potential pathogenicity. Encouragingly, none of the strains demonstrated. Hemolytic activity was observed in all LAB isolates, raising concerns about potential pathogenicity. Encouragingly, none of the strains demonstrated gelatinase activity, neither biogenic amine production, suggesting a reduced risk of toxic amine accumulation in matured cheeses. Bacteriocin production tests indicated that some Enterobacteriaceae strains exhibited inhibitory effects against *Listeria monocytogenes*. Antimicrobial susceptibility profiling revealed concerning levels of antibiotic resistance among strains historically linked to bovine mastitis



(Enterococci), emphasizing the need for stringent hygiene practices and improved farm management strategies. Our study underscores the need for scientifically guided interventions in artisanal cheese production to enhance production practices. One approach is to locally screen for safe starter cultures, or to use standardized microbial consortia made with native strains tested for safety. Such strategies could reduce microbial risks while preserving Brazil's cheese heritage. Our findings advance understanding of microbial dynamics in cheese producing environments and highlight the role of microbiological innovation in ensuring food safety and sustainable production.

References

- BLAYA, J. BARZIDEH, Z.; LAPOINTE, G. Interaction of starter cultures and nonstarter lactic acid bacteria in the cheese environment. *Journal of Dairy Science*. v. 101. p. 1-19. 2018.
- KAMIMURA, B. A.; MAGNANI, M.; LUCIANO, W. A.; CAMPAGNOLLO, F. B.; PIMENTEL, T. C.; ALVARENGA, V. O.; PELEGRINO, B. O.; CRUZ, A. G.; SANT'ANA, A. S. "Brazilian Artisanal Cheeses: An Overview of Their Characteristics, Main Types and Regulatory A
- Mladenović, K.G., Grujović, M., Kiš, M., Furmeg S, Tkalec VJ, Stefanovic OD, Kocić-Tanackov SD. 2021. Enterobacteriaceae in food safety with an emphasis on raw milk and meat. *Appl Microbiol Biotechnol* 105, 8615–8627 (2021).
- OLIVEIRA, D. P. Caracterização funcional genômica dos micro-organismos predominantes no fermento endógeno "pingo" do queijo da Serra da Canastra. 2020. Dissertação (Mestrado em Ciência dos Aliment

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PP075: Are you having trouble to characterize *Bacillus* spp.? You should read this

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After uncovering misidentifications of four among six strains of *Heyndrickxia coagulans* received from different microbial banks, a review of existing methods led us to develop and propose a microbiological characterization protocol aimed at minimizing errors and improving accuracy in *Bacillus* species-based products, such as probiotics, thereby enhancing the quality, safety and reproducibility of those products and techniques.

Six strains (A1,A2,B1,C1,D1,D2) presumed to be *Heyndrickxia coagulans* were obtained from four different banks (Banks: A,B,C,D). Cultures of all strains were activated according to the recommendations for *Bacillus* spp. (Poormontaseri et al.,2017). Identification by MALDI-TOF and partial genome sequencing on 16S rRNA for all strains (A1,A2,B1,C1,D1) and Whole Genome Sequencing (WGS) for strains A1 and A2 were performed.

Both identification approaches (MALDI-TOF and 16S-rRNA) revealed that strains A2 and B1 belong to species *Heyndrickxia coagulans*, A1 and D2 to *Bacillus subtilis*, C1 to *Bacillus cereus*, and D1 to *Cytobacillus oceanisediminis*.

MALDI TOF identification is considered as are highly reliable, simple, fast and provided results with scores between 2.0 and 2.3, that indicate highly probable genus identification and probable species identification (Topić Popović et al., 2023). Despite the limitations and challenges existent for the taxonomic identification through 16S-rRNA sequencing method due to the intragenomic variations in the conserved regions of *Bacillus*-related species and high interspecies similarity of their 16S-rRNA gene sequences that can lead to inaccurate identification of closely related strains or species, our results were consistent with those obtained from MALDI-TOF, reinforcing precision of our strain identification. To address the limitations of 16S-rRNA sequencing for characterizing *Bacillus*-related species, further analysis through WGS or even 16S-rRNA next-generation sequencing is considered as relevant step in the appropriate identification, becoming even standard for strains with applications in fermentation processes or putative probiotics (Rizal et al., 2020). In relation to strain A1, based on all preliminary biochemical and physiological tests, MALDI-TOF, 16S-rRNA and WGS was confirmed that strain belong to *Bacillus subtilis* and not *Heyndrickxia coagulans* (Uono et al., 2019). Following the consequences of the experience with strain A1, facts of time- and budget-consuming experience, the team decided to prioritize MALDI-TOF analysis and 16S-rRNA sequencing before WGS. By employing a combination of morphological, biochemical, MALDI-TOF, and 16S-rRNA analyses, we established a more reliable and comprehensive protocol to ensure precise identification of *Bacillus*-related species. The findings demonstrate



that while MALDI-TOF and 16S-rRNA sequencing are valuable tools for initial steps for accurate and cost-effective pre-identification of *Bacillus*-related genera, they must be complemented by more definitive methods such as WGS for conclusive results. Morphological, biochemical, such as the simple catalase test and physicochemical analysis should be conducted after the preliminary confirmation through MALDI-TOF and 16S-rRNA sequencing for the better species characterization (Vos et al., 2011). This layered approach not only minimizes errors but also enhances the safety, quality, and reproducibility of *Bacillus*-related species-based products and techniques. Implementing this refined protocol can significantly reduce the incidence of misidentification, ensuring that industrial and probiotic applications of bacterial strains are both effective and safe.

References

- Poormontaseri, M., Ostovan, R., Berizi, E., & Hosseinzadeh, S. (2017). Growth rates of *Bacillus* species probiotics using various enrichment media. *International Journal of Nutrition Sciences*, 2(1), 39-42
- Topić Popović, N., Kazazić, S. P., Bojanić, K., Strunjak-Perović, I., & Čož-Rakovac, R. (2023). Sample preparation and culture condition effects on MALDI-TOF MS identification of bacteria: A review. *Mass spectrometry reviews*, 42(5), 1589-
- Rizal, N. S. M., Neoh, H. M., Ramli, R., Hanafiah, A., Samat, M. N. A., Tan, T. L., ... & Khor, B. Y. (2020). Advantages and limitations of 16S rRNA next-generation sequencing for pathogen identification in the diagnostic microbiology laboratory: pers
- Uono, M. T., Hacker, S. S., Manfrinato, C. V., Matsuo, M. M., Todorov, S. D., & Bogsan, C. S. (2019). Technological Development of Probiotic Supplement for Zootechnical Improvement of Broilers. *Journal of Advanced Agricultural Technologies Vol*, 6(1).

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