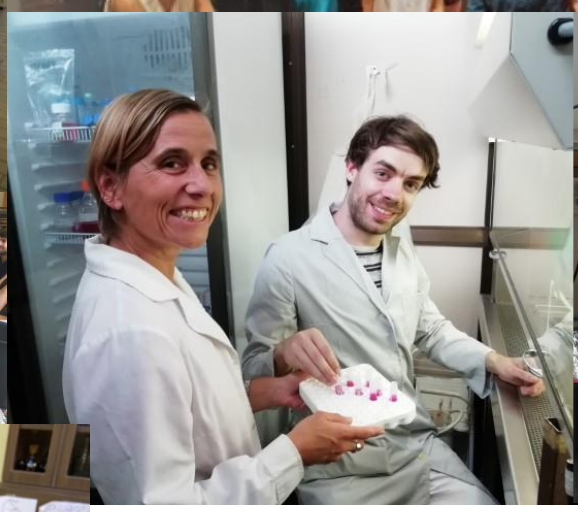
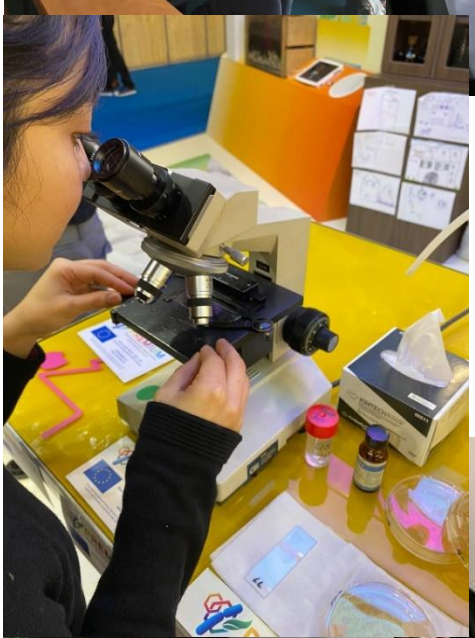
PREMIUM

Preserving bacteria with oligosaccharides and eco-friendly processes





PREMIUM Newsletter #1

March 2020



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EDITO

Started in January 2018, the project PREMIUM has now reached its midterm. This newsletter is the occasion to present the progress of project activities in terms of science and exchanges among partners.

During the first two years of PREMIUM project, information, experience and skills were exchanged among all partners, both internationally and across sectors, thus leading to the enrichment of knowledge on the different topics covered by PREMIUM. This was done through secondments, face to face meetings, co-supervision of PhD students, training sessions and collaborative dissemination of results.

Kick-off meeting – Paris, March 2018

Project meeting – Argentina, March 2019

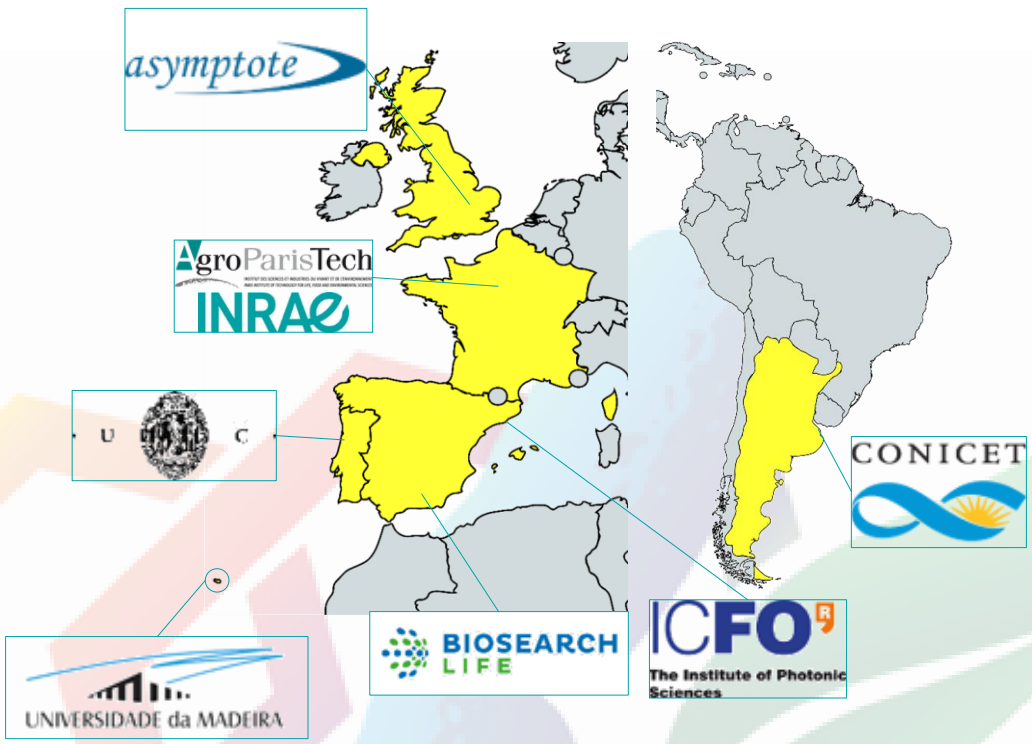
Mid-Term meeting – Paris, June 2019

CONTENT

PREMIUM

Preserving bacteria with oligosaccharides and eco-friendly processes

Project partners and fields of expertise



- **Food microbiology and process engineering:** INRAE, AgroParisTech, Biosearch, CONICET
- **Biochemistry:** UMa, CONICET, AgroParisTech, INRAE, UC
- **Life cycle assessment and multi-criteria analysis**: INRAE
- **Cryobiology and biophysics:** Asymptote, INRAE
- **Molecular Dynamics:** UC
- **Vibrational spectroscopy and imagery**: ICFO, INRAE, CONICET
- **Multivariate analysis**: ICFO, CONICET

INRAE – Institut national pour l'agriculture, l'alimentation et l'environnement www.inrae.fr
AgroParisTech – Institut des sciences et industries du vivant et de l'environnement www.agroparistech.fr
UC – Universidade de Coimbra www.uc.pt
UMa – Universidade da Madeira www.uma.pt
ICFO – Fundacio Institut de ciencies fotoniques www.icfo.eu
Asymptote Ltd www.asymptote.co.uk
Biosearch S.A. www.biosearchlife.es/en
CONICET – Consejo Nacional de Investigaciones Cientificas y Tecnicas www.conicet.gov.ar

PREMIUM

Preserving bacteria with oligosaccharides and eco-friendly processes

Why PREMIUM?

Fermentation is at the origin of many food products: yogurt, cheese, bread, wine, etc. It is essential to the food-processing industry.
Behind the fermentation process, are microorganisms called lactic acid bacteria or LAB. The current industrial processes applied for producing and preserving bacteria generate important changes in their natural environment and induce cell death and losses in fermentation properties (functionality). The use of oligosaccharides is a way of increasing their resistance. Far from being optimised, the process of LAB preservation causes significant loss and over-consumption of energy.

Microorganisms are unicellular organisms which are microscopic, not visible with naked eye. They include bacteria, fungi, archaea, protists and viruses, and are among the earliest known life forms. In **PREMIUM** project, we are interested in microorganisms that are used for producing fermented foods and present benefits for human health.

Lactic acid bacteria produce large amounts of lactic acid from carbohydrates, but also organoleptic compounds (flavours, exopolysaccharides) and substances limiting the growth of other microorganisms (bacteriocins). Some LAB present probiotic properties (health benefits). In **PREMIUM** project, we study LAB exhibiting different resistance to preservation processes.

Oligosaccharides (FOS and GOS) are short chain carbohydrates naturally present in plants and are considered as dietary fiber. They promote the growth in the gut of bacteria with beneficial health properties.

During the first two years, **PREMIUM** partners developed new and improved existing methods for producing oligosaccharides and stabilised bacteria.
Progress was also made in the understanding of bacteria protection mechanisms during stabilisation processes and in the transfer of knowledge and methods to improve the preservation of other cells (namely, mammalian cells).

Stabilisation processes refer to means of increasing product shelf life until final usage for fermentation process. The strategy consists in reducing water availability thus limiting microorganisms growth and chemical reaction. Water can be converted into ice (freezing) or removed from the product (drying).

Sustainability focuses on meeting the needs of the present without compromising the ability of future generations to meet their needs. It is composed of three pillars: economic, environmental, and social. In **PREMIUM** project, we are focusing our research mainly on the environmental pillar: identifying environmental hotspots of the whole system of production and stabilisation of LAB and comparing different scenarios for minimising the environmental impact.

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Scientific objectives of PREMIUM

Lactic acid bacteria

Starters* market increases
*concentrated cultures of bacteria

But low efficient processes

A large variety of functionalities offered by microorganisms remains under-exploited due to their sensitivity to the manufacturing processes.

The **objective of PREMIUM project is to develop new strategies to preserve lactic acid bacteria from laboratory to industrial scale**

High functionalities
Exploit new microorganisms

Innovative processes
High productivity

Low environmental impacts

Production, characterization and screening of oligosaccharides (WP2)

- Enzymatic synthesis
  Hydrolysis of plant biomass
  > 20 compounds
- Protective ability to preserve bacteria during stabilisation processes
- Life cycle assessment

Selection of 4 oligosaccharides

Development and optimisation of stabilisation processes (WP3)

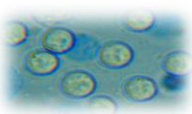
- Bacteria encapsulation
- 5 stabilisation processes
- Multicriteria analysis

Innovative and sustainable processes

Elucidation of protective mechanisms of oligosaccharides (WP4)

- From the protective matrix
- Bacterial cells
- To bacterial membrane

Transfer to industry and to other microorganisms and cells (WP5)



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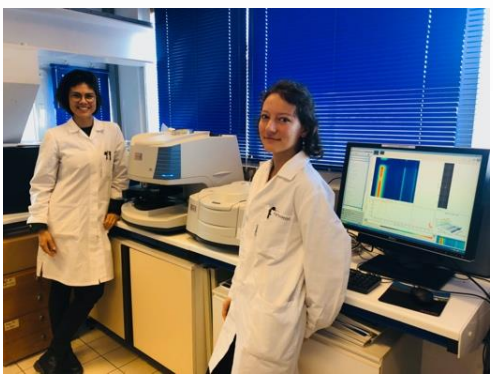
Preserving bacteria with oligosaccharides and eco-friendly processes

PREMIUM is a 4-year staff exchange project between 5 **academic** and 2 **industrial** partners. These exchanges also called **Secondments** are implemented throughout the project allowing development of research **collaboration between partners and knowledge transfer**. Secondees are partners staff counting Experienced Researchers, Early-Stage Researchers (PhD students) and technical staff.
The secondments carried out for the first two years of the project represent a total of **74 months** spent by secondees at partners site.

FTIR microspectroscopy on lactic acid bacteria under freezing stress

Transferring know-how involved training on the experimental set up, software utilisation and data treatment. The final aim was to find out markers of freezing stress During her secondments at INRAE in 2018 and 2019, Julie Meneghel from Asymptote (UK) trained Amélie Girardeau (INRA PhD student) on how to perform FTIR measurements with the IN10 FTIR microscope. Main experiments were done on Lactic Acid Bacteria under osmotic stress.



Screening cryoprotective effect of some new FOS and GOS produced by synthesis and hydrolysis on Jurkat cells

In January 2019, Paula Castilho from University of Madeira (UMa, Portugal) worked with Julie Meneghel and Peter Kilbride (Asymptote) at testing three samples of fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) produced by synthesis as well as two GOS samples produced by hydrolysis, for their ability to protect Jurkat cells during freezing.

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Preserving bacteria with oligosaccharides and eco-friendly processes

Secondments

"During my secondment in March 2019 at CONICET (Argentina) and with the help from Ayelen Hugo, I tested new sugars (developed for bacteria cryopreservation) on mammalian cells. With the hope that the new knowledge generated in the project could drive further human medical advances." – Peter Kilbride (Asymptote, UK)

"During my secondment at ICFO, in collaboration with Monica Marro (ICFO, Spain), I did measurements of Raman spectra from fructooligosaccharides (FOS). This substances are very important in protection lactic acid bacteria during stressful process." – Esteban Gerbino (CONICET, Argentina)

"During my secondment at AgroParisTech (France), I gained experience in control and analysis of the physical properties of cryoprotected biomass as well as acidifying activity measurement in different stages of the production process." – Sonia Campoy (Biosearch Life, Spain)

"During my secondment at CONICET I prepared the liposomes used for testing the prebiotics developed for bacteria. With the hope that the new knowledge generated could drive further advances in food quality." – Marie-Hélène Ropers (INRAE, France)

"During my secondment at CONICET, I trained students on bioprocessing and fermentation of bacteria starters. I could also define laboratory facilities for fermentation." – Catherine Béal (AgroParisTech, France)

"During my secondment at AgroParisTech, with Caroline (INRAE) and Camille (APT) we performed Life Cycle Assessment of prebiotics production." – Esteban Gerbino (CONICET, Argentina)

"During my secondment at INRAE I investigated the impact of process conditions on physical and biological properties of two industrial Lactobacillus bacteria." – Maria Guerrero (Biosearch Life, Spain)

"I gave lectures at CONICET on membrane lipids and proteins, and lipid droplets. I also made research on characterization and properties of membrane lipids of lactic acid bacteria." – Yann Gohon (AgroParisTech, France)

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Networking and project activities

Knowledge sharing, interdisciplinary and intersectoral dialogue, new skills acquisition and career development are at the core of PREMIUM. During the first two years of the project, numerous activities were organised in addition to secondments counting around 210 participants. Following is a sample of project activities held in 2018.

Project kick-off meeting in Paris, March 2018

The 1st annual PREMIUM meeting was held in Paris by coordinator INRAE and marked PREMIUM's kick-off. This meeting was the opportunity for project partners to meet in person and plan the implementation of project activities and secondments.

Seminar on Preservation of Lactic Acid Bacteria in Granada, April-May 2018

As an example of knowledge sharing among project partners, the two seminars on Preservation of Lactic Acid Bacteria performed by INRAE and AgroParisTech (2018, April 25-26 and May 2-4) were validated by project partner Biosearch as an official training action for their employees. Over 75 of Biosearch employees attended the seminar.

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Project meeting and workshop in La Plata, March 2019

A follow-up on project activities was organised in La Plata, Argentina, by project partner CONICET in March 2019. This permitted "live" discussions on PREMIUM's results through technical presentations, as well as a fine-tuning of secondments planning.

Project meeting was followed by a 1-day workshop on Prebiotics and was attended by over 40 participants both from the project and external (post-graduated and graduated students).

Summer School in La Plata, March 2019

This training represented 60 hours of teaching and was validated as official training for the 8 students who attended. This was organised at La Plata (Facultad de Ciencias Exactas) by AgroParisTech and lead by teachers from AgroParisTech, INRAE and UC. Three modules were proposed on: biotechnology and bioprocess engineering (30 hrs), biochemistry and biophysics of lipid membranes (15 hrs) and functionalities of microbial ecosystems of traditional fermented foods (15 hrs). The theory was completed with practical sessions on the production of LAB.

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Workshop on Process eco-design: application on Lactic Acid Bacteria preservation in Paris, June 2019

This project workshop organised by INRAE and AgroParisTech aimed at understanding challenges of an eco-design approach for a company. Exercises in working groups were conducted to test the approach in the case of production of Lactic Acid Bacteria stabilised by using oligosaccharides and to imagine strategies for sustainable design of stabilised LAB.

Outreach of the project – Key data

8 peer-reviewed articles
2 book chapters
6 poster presentations
8 oral presentations
2 invited lectures

PREMIUM website
3 social media
1 press release
1 promotional video

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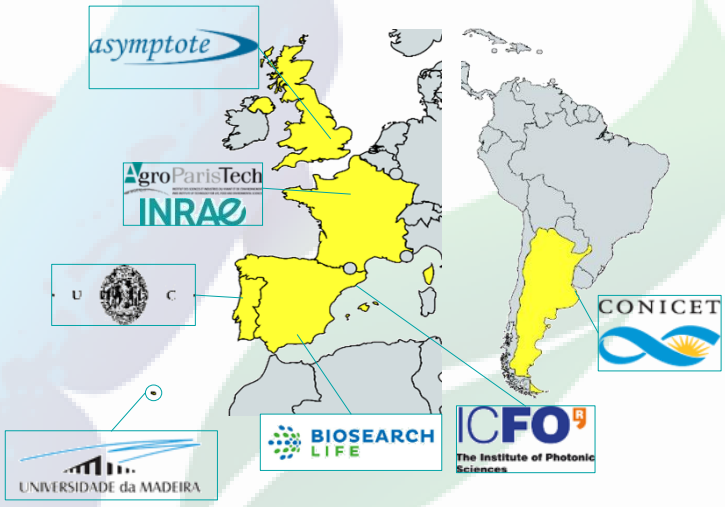
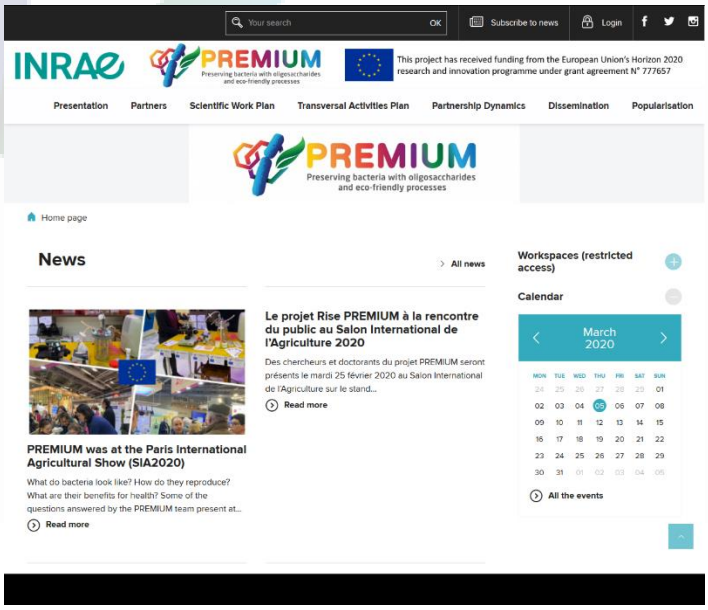
Project partners at Mid-Term Meeting with EC representative, Irina Tiron (top right) – Paris, June 2019

PREMIUM is a four-year staff exchange **multidisciplinary** program between 5 **academic** partners of three European countries (France, Portugal and Spain), one non-European country (Argentina) and 2 European **industrial** partners (Spain and United Kingdom).





Find out more about PREMIUM at https://www6.inrae.fr/premium/

Next issue in September 2020

Check our website:
https://www6.inrae.fr/premium/

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PREMIUM RISE

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