







# MICROBION

**Open Innovation in Agri-Food Microbiology** 

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# WHO WE ARE

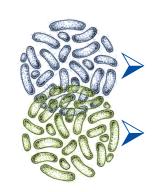
Microbion is a CRO providing microbiology R&D services to







the **best performing** microbes (strains) for each application, through innovative **DNA-based methods**.



Problem solvers
Innovation enablers













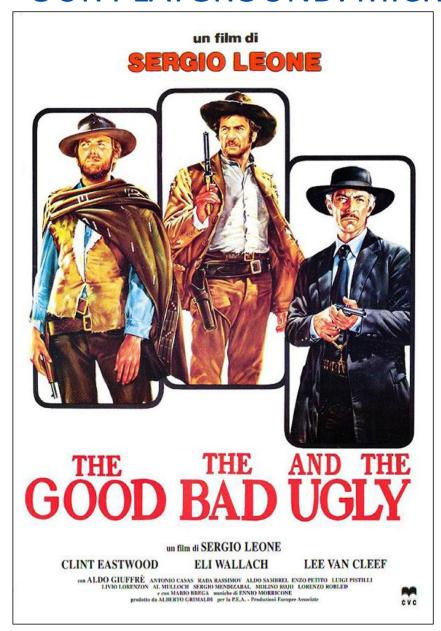




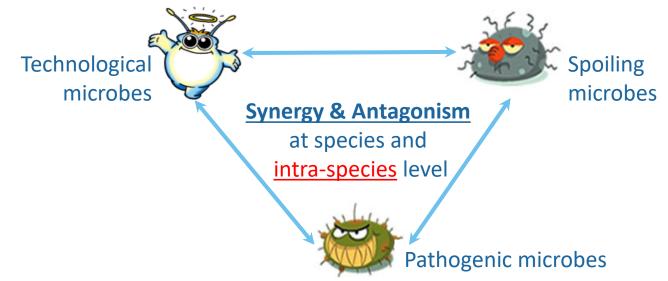




# **OUR PLAYGROUND: MICROBIAL BIODIVERSITY**







# **OUR STORY**



Spin-off Verona University

2011



1<sup>st</sup> business partner

2012



1<sup>st</sup> Strain licenced

2014



1<sup>st</sup> Patent deposit

2015



1<sup>st</sup> EU project + EIT Food acceleration

2017





















# **OUR STORY**



Spin-out from campus

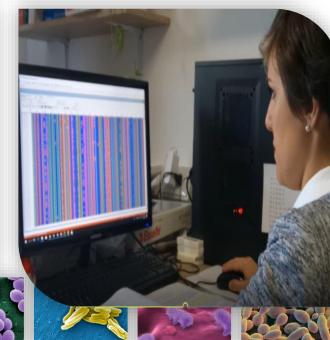
2018

- √ 300 m<sup>2</sup> labs
- √ 5 lab technician
- √ 4 R&D managers
- √ 3 freelance specialists
- ➤ Microbiology lab (BSL2)
- ➤ Molecular biology lab
- ➤ Bio-banking unit
- > Bio-informatics unit
- > 60 customers served> 1 M€ service sales(cumulative)







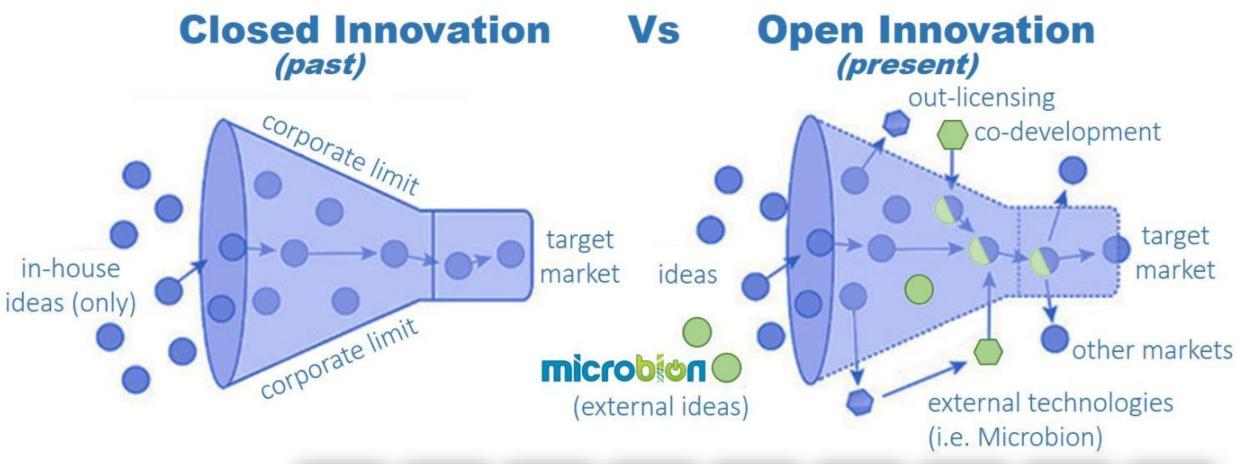








# THE OPPORTUNITY























# **OUR PLAYGROUND: MICROBIAL BIODIVERSITY**



Biofilms, spores, heat-resistantEmerging resistant strains

Spoilage, outbreaks, RCA Batch val. & Product shelf-life

### **Food Safety**

Product stability Challenge tests

#### **Process Validation**

Risk assessment Genomics of Antibiotic Resistance

#### Health

Identification, bioindicators
Detection, rapid & portable dev.

**Molecular Diagnostics** 

Industrial microbes

- Selected, manufactured and applied
- Controlled spontaneous fermentation

Bio-fertilisers, biostimulants Bio-pesticides, biocontrol

### Agriculture

Cultured food & beverages
Spontaneous Fermentations

#### Food

Probiotics, pre/post-biotics Microbiota in-depth analysis

#### Health

Bio-fuels, WWTP, bio-plastics Precision Fermentation

**Green Chemistry** 

We provide insight and practical solutions for any application

















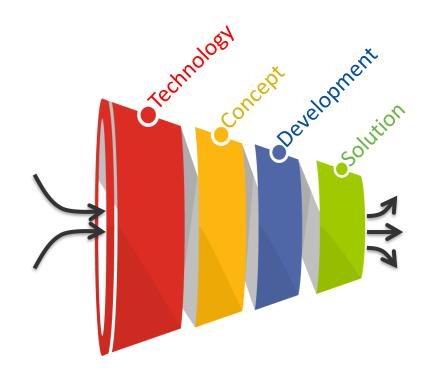




## **OUR KNOW-HOW AT WORK**

#### **Know-how:**

- → Molecular microbiology
- → Species identification
- → Strain characterization
- → Strain authentication
- → Bio-banks screening
- → Genome analysis
- → Comparative genomics
- → Metagenomics
- → Culturomics
- → Patents expertise
- → Agri-Food & Pharma Regulatory



### Services provided:

- ✓ Starter selection
- ✓ Strain tracking
- ✓ Fermentation monitoring
- ✓ Safety assessment
- √ Strain manufacturing & quality control
- ✓ Microbial stability tests
- √ Microbiota analysis
- ✓ Challenge-tests of product/plant
- ✓ Bio-banks of contaminants/strains
- ✓ Strain-specific diagnostics
- ✓ Intellectual Property Rights
- ✓ Business intelligence
- √ Regulatory Compliance





















## INNOVATIVE STRAINS SELECTION

A practical example of one of our projects

#### The problem:

Amarone wine is made from 2 months dried grapes

→ only 2 strains <u>commercially available</u> can work at this sugar/alcohol concentration ranges

#### The solution:

- Bio-based fermentation optimisation
- Regulatory compliance (DOC)
- •Distinctive traits and improved quality wines









MASI Yeast Bio-Bank (100+ yeasts)



Experimental wine production (10+ yeasts)



Scale-up winemaking (2 yeasts)

*S. cerevisiae* & Non-Saccharomyces



**Distinctive flavours** 

Cost saving & less CO<sub>2</sub> footprint

**Seasonal consistency** 

Unique on the market!















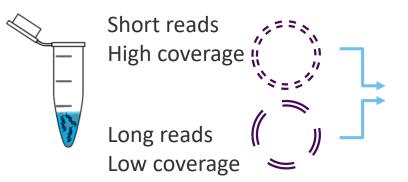




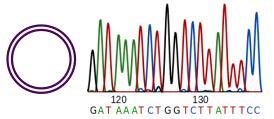




## **COMPARATIVE GENOMICS PIPELINE**



Hybrid assembly (1-scaffold genome goal)



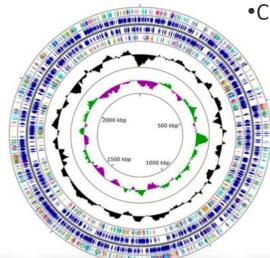
WG Mapping barcoding & assembly validation (1-scaffold genome goal)



- Plasmids characterization
- Gene functional annotation
- KEGG pathway & COG/KOG classification
- •Custom CDS genome maps



- ■Element AVITI
- ☐NovaSeq Illumina
- ☐MiSeq Illumina
- ☐Oxford Nanopore



High Quality
Genome Sequence

(validated by independent technology)















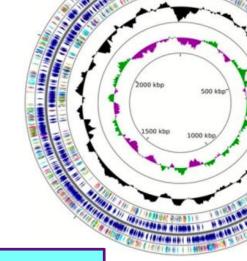






# **COMPARATIVE GENOMICS PIPELINE**







- Pests antagonism
- Adhesions genes
- Short chain fatty acids
- Bacteriocins
- C/N metabolisms
- Mechanism of action genes



- Virulence genes
- Antibiotics resistance
- Biogenic amines
- Antigens
- Bacteriophages
- Horizontal gene transfer



- Plasminds
- Diagnostics biomarkers
- Field trials tracking
- Genome stability
- Differentiation from prior patented strains













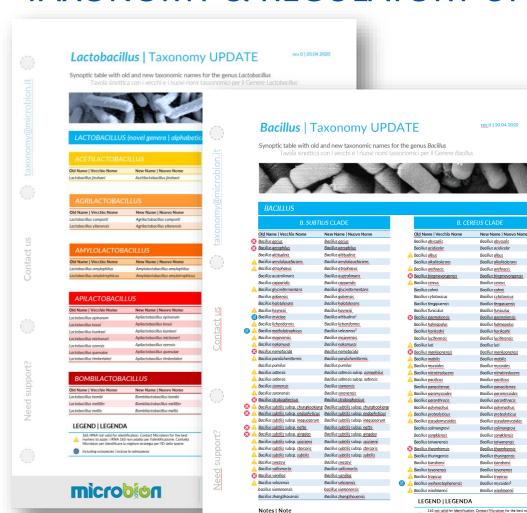








# **TAXONOMY & REGULATORY UPDATE**







Based on: Patel and Gusta (2020) Int. J. Syst. Evol. Microbiol. 2020;70:406-438

Liu Y, Lai Q, Du J, Shao Z. (2015) let J Syst Evol Microbiol 2015; 65:2769-2773. Dunlage et al. (2016) let J Syst Evol Microbiol 2016 Marsh631:1212-1217 Liu Y, Lai Q, Shao Z. (2016) let J Syst Evol Microbiol 2018; 68:106-112. | Gen A, Garrity GN (2018) let J Syst Evol Microbiol 2018; 68:106-112.

See notes | Vedi note

Not validly published/rejected name| Nome non validate



EFSA Journal 2011;9(3):2117

#### SCIENTIFIC OPINION

#### Guidance on the assessment of microbial bi

EFSA Panel on Additives and Products or Substances

European Food Safety Authority (EFSA

#### BACKGROUND

Directive 82/471/EEC4 regulated the use of certain products us direct or indirect protein sources. Included in these certain prod fermentation of different substrates by microorganisms. The a value of these products was done according to the guidelines fix

In 2009, the European Parliament and Council adopted Regula on the market and use of feed, which repeals both Directives September 2010. Biomasses produced from non-genetically mo authorisation/assessment to be placed on the market. However, those biomasses which are produced from a genetically modified

In recent years EFSA has received several dossiers related market of biomasses obtained from the production of amino a applications fall under the scope of Regulation (EC) No 18 genetically modified microorganisms. Applicants have been req by the GMO Panel8 for the assessment of the genetic modif Directive 83/228/EEC for the assessment of the product itself. now been repealed, are more than 25 years old and the requir assessment practices of the FEEDAP Panel.

Therefore, there is a need for an up-to-date guidance document dossiers for the assessment of biomasses for use in animal nut nutritional value and safety of the product itself but not modification, which are covered by the guidance of the GMO Pa

- On request of EFSA, Question No EFSA-Q-2010-00939, adopted on 16 Ma Panel members: Gabriele Aquilina, Georges Bories, Andrew Chesson, I Albert Dierick, Mikolaj Antoni Gralak, Jürgen Gropp, Ingrid Halle, R Lundebye Haldorsen, Alberto Mantovani, Miklós Mézes, Derek Rei FEEDAP@efsa.europa.eu
- Acknowledgement: The Panel wishes to thank the members of the Workin and Atte von Wright, for the preparatory work on this scientific opinion.
- OJ L 213, 21.7.1982, p. 8.
- OJ L 126, 13.5.1983, p. 23.
- OJ L 229, 1.9.2009, p. 1. OJ L 268, 18.10.2003, p. 1.
- http://www.efsa.europa.eu/en/scdocs/scdoc/374.htm

Suggested citation: EFSA Panel on Additives and Products or Substances use isment of microbial biomasses for use in animal nutrition. EFSA Journal doi:10.2903/j.efsa.2011.2117. Available online: www.efsa.europa.eu/efsaiou

© European Food Safety Authority, 2011

#### WHAT IS AN INTERNATIONAL PROTOCOL?



Users may be corporations, laboratories, research centers, individuals, indigenous peoples and local communities interested on utilizing genetic resources to produce foods, medicine, cosmetics or biochemical procedures based on biotechnology and research of the genetic information in plants, animals, microorganisms, fungi and any other sort of being containing genetic information and their derivatives

The Protocol acknowledges the rights of indigenous peoples and local communities to share the benefits arising from utilization of genetic resources and associated traditional knowledge; they have been key actors for the preservation of biological diversity based on their knowledge, practices and experience accumulated through centuries and handed down from one generation to another.

Furthermore, the protocol is a scientific research incentive guaranteeing legal certainty within an international framework since it seeks to avoid misappropriation and illegal practices by establishing clear and transparent mechanisms to access genetic resources and associated traditional knowledge.



#### OALS OF THE UNOP-SEMARNAT PROJECT



To have a legal frame in Mexico and to establish administrative or public policy measures to regulate access and utilization of genetic resources and associated traditional knowledge thus guaranteeing fair and equitable benefit sharing.





To create a biodiversity value-oriented culture among relevant actors as a base for preservation and sustainable use of genetic resources and associated traditional

To strengthen institutional

capacities related to the

Nagova Protocol.

To protect genetic resources and associated traditional knowledge and to foster capabilities related to that protection within indigenous peoples and local communities.



To integrate universities, research centers, civil society organizations, private initiative and society overall in the project implementation













# REGULATORY COMPLIANCE

**Experience with international regulatory authorities:** 

EFSA (EU)

EMA (EU)

FDA (USA)

EPA (USA)

SAHPRA (South Africa)

CFIA (Canada)

MFDS-KFDA (Korea)

**Experience in compliance of product categories:** 

Agro-chemicals

Food/Feed Ingredients

Food/Feed Additives

Food/Feed Supplements

**Medical Devices** 

**Drugs (Live Biotherapeutic Products)** 

**Experience with the demonstration process:** 

Genome-bases safety assessment

**QPS** status

**GRAS** status

Field Trials for claims substantiation

Clinical Trials for claims substantiation

Adopted: 28 June 2024

DOI: 10.2903/j.efsa.2024.8912

STATEMENT



EFSA statement on the requirements for whole genome sequence analysis of microorganisms intentionally used in the food chain

**European Food Safety Authority (EFSA)** 

Correspondence: feedap@efsa.europa.eu

Abstract

Microorganisms, genetically modified or not, may be used in the food chain either as active agents, biomasses or as production organisms of substances of interest.

Microbion is among the panellist for new standard for genome sequencing and analysis!











# INTELLECTUAL PROPERTY RIGHTS

#### **Business Intelligence services:**

- ✓ collect strains from IDA bio-banks anonymously
- ✓ compare your strains with competitors and with prior-art strain
- ✓ technology scouting and market research
- ✓ competitive advantage analysis

### Patent filing services (microbes and/or their application):

- ✓ prior-art search
- √ requirements check
- ✓ IDA compliant deposit
- ✓ technical description to the patent attorney
- ✓ reply to evaluators with the patent attorney

#### **Examples of patents registered by our customers:**

**EP3194565A2** - Strain of the yeast species *Saccharomyces cerevisiae*, strains essentially derived from it and use thereof **WO2019123329A1** - Composition for use in the treatment and/or improvement of sleep and mood disorders (probiotics) **ITUB20152796A1** - Ceppo di *Saccharomyces cerevisiae* CS1, sue composizioni e suoi usi























We can collaborate with your favourite patent attorney, or we can offer turnkey service together with N&G law firm

# **OUR TECHNOLOGIES**

#### **MICROBIOLOGY MOLECULAR BIOLOGY INNOVATIVE TECHNOLOGIES** ■ Plate counts ☐ End-point PCRs ☐ Whole genome sequencing ■ Antagonism assays ☐ Fingerprinting PCRs (long reads & short reads) Phenotypic assays ☐ Real-Time PCRs ☐ Bioinfomatics ☐ Antimicrobials resistances ☐ Quantitative PCRs ☐ Transcriptomics ☐ Fermentation ☐ Viability PCRs ■ Metabarcoding ☐ Stability-tests ☐ LAMP-PCRs Metagenomics Lyophilization ☐ Multi-locus sequence typing ☐ Flow Cytometry Cryopreservation ☐ In-situ FISH ☐ Infrared spectroscopy ■ Microscopy Optical mapping ■ MALDI-TOF ..and more ...and more ...and more





















# **CHALLENGE TEST 2.0**

**Classic Challenge-test** 

Buy quantified reference contaminants

Inoculum

Verify efficacy

**Challenge-test 2.0** 

**Raw Materials** 

Contaminants bio-bank

Production and inoculum of **native contaminants** 

- **Equipment sanitization**
- **✓** Pasteurization temperature
- **Storage conditions**
- ✓ Shelf-life
- **Packaging barrier**
- **Preservatives**

**More reliable** modelling!



















# **OUR EXPERTISE**



Microorganisms
Identification



Challenge Tests



Microorganisms Characterization



Technical Problem Solving



Microorganisms Validation



IPRs & Regulatory
Support





















# **OUR MARKETS**

#### **Best-selling services:**

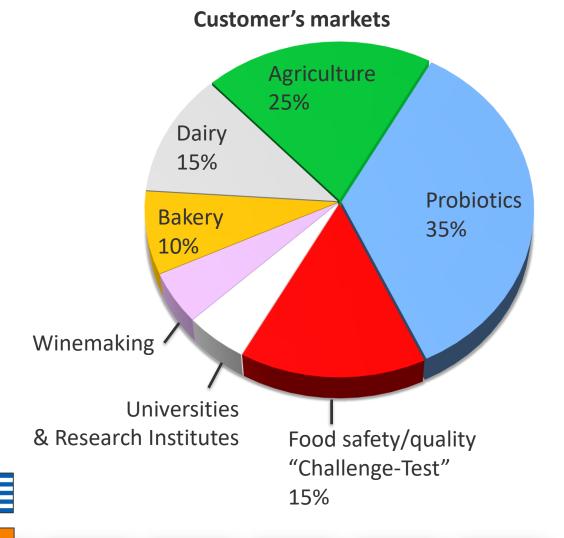
- ✓ Genome-based safety assessment reports
- ✓ Customised molecular analyses (PCRs)
- ✓ Strains selection and characterization
- ✓ Food Challenge-Tests & cleaning validation
- ✓ Open Innovation microbiology services

#### Our products:

- ✓ SAFEbion-V (SARS-CoV-2 surrogate)
- Biological Standards for validation of microbial load reduction and sanitization

#### International markets served:















# MICROBION PROPRIETARY TECHNOLOGY (HPME)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau

(43) International Publication Date 25 January 2018 (25.01.2018)





(10) International Publication Number WO 2018/014979 A1



Highly polymorphic and modular extragenic (<u>HPME</u>) markers within specific taxa of microorganisms and use thereof for their differentiation, identification and quantification:

- •TRL 9 Probiotics application
- •TRL 8 Agriculture bio-control application
- •TRL 7 Fermented foods application

Validated differentiation of all species & subspecies of:

- ✓ Lactobacillus
- ✓ Bifidobacterium
- ✓ Bacillus
- ✓ More genera with HPMEscan

# Distinguish genetically close-related microbes even in mix Solve the problem of very similar 16S rRNA sequences!

- Microbion unique services
- License for diagnostic devices























# Transparency solutions for transforming the food system



Horizon Europe HORIZON-CL6-2021-FARM2FORK-01-17 Grant Agreement 101060739

September 2022 – September 2026 Budget € 11M 27 Partners



www.titanproject.eu



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# **PROJECT CONCEPT**

Platform for innovations that aid transparency to make the food system **fair**, **healthy**, and **environmentally friendly**.

- Food system is highly fragmented
- Claims are difficult to be verified

#### 15 innovative solutions

(prototype to market launch)

- DNA-based Rapid Methods
- Blockchain
- Artificial Intelligence
- Internet of Things



#### 3 Domains

- Safety
- Sustainability
- Health

Next-Generation-Sequencing (+ LAMP-PCR)





Industrial microorganisms

Microbial contaminants





UNIVERSITÀ CATTOLICA del Sacro Cuore

To check presence and viability of species/strains claimed

To identify and detect emerging contaminants of new plant-based food

# **STATE OF THE ART**

#### **Huge potential of NGS**

- Portable NGS devices
- Easy to use
- Inexpensive
- Fast protocols

## Metabarcoding based on 16S rRNA

- Universal marker (universal PCR primers)
- Comprehensive public databases for match
- Identical in close related species
- Great for research (not for QC)

## Metabarcoding based on **HPME markers**

- Genus-specific markers (multiplexable)
- **Hyper variable regions** (sequence & length)
- Resolve close related species/subspecies/strains
- Match with Microbion database (international patent WO2018014979A1)



AGCTCGTTCCGGATCGGCATGCATTGATACGCT
AGCTCGTGCATGCGAGGCATGCATAGCTCGATACGCT
AGCTCGTCCCGGATGGGCATGCATCAATAGATACGCT

Conserved Variable Conserved (PCR primers) (informative) (PCR primers)

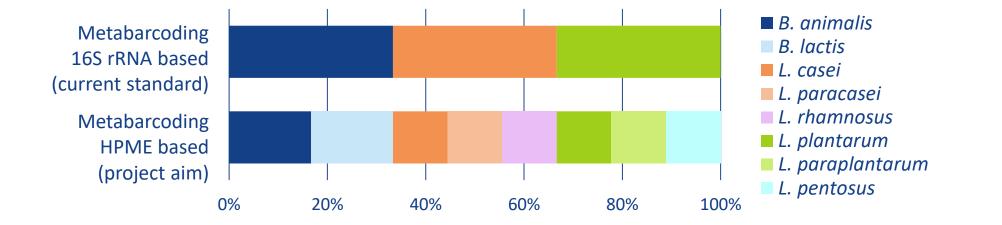
AGCTCGTGATCG--ATC---ATCGCAGGCGCAGCTTCGCGATACGCT

AGCTCGTCT--CGATC--GC----CAAA-----GTACCGATACGCT

# FIRST DELIVERABLE







NEW SERVICE!
Flow Cytometry + NGS
Enumeration and identification within 24 hours

- ✓ Probiotics
- √ Food Cultures
- ✓ Biocontrol & Biostimulants

# EIT FOOD PAST PROJECTS



INNOPOULTRY BP2018

New feeds for poultry industry

**→** Probiotics

➤ Microbiome improvement

click here to know more



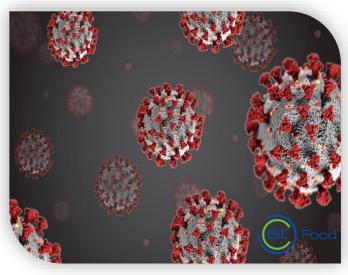
FISH TRUST BP2018 & 2019

Portable device for fish fillets quality & safety

➤ Microbial load

➤ Listeria detection

click here to know more



**COVID-19 BEAM IT UP** *BP2020* 

SARS-CoV-2 model to validate detection and sanitization products and protocols

➤ Surrogate virus

➤ Portable, fast, easy-to-use diagnostic device

click here to know more





















# PIMENTO COST Actions (2021-25)





# Promoting Innovation of ferMENTed fOods

- 200+ experts:
  Universities, Industries, NGOs
- Main WPs:
  - Cartography of Fermented Foods (FF)
  - Health benefits and risks of FF
  - Federating scientists and FF producers to boost innovation for society (co-leader)
- Microbion's role:
  Research-Industry connector and catalyser

Click here to know more







- ✓ Survey on bottle-necks of innovation in FF
  - Regulatory
  - Food Safety
  - > Tech & Processing
- ✓ Position paper to EU Commission
- ✓ Partnership with EFSA for guidelines
- ✓ Association of the European FF industry























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# **THANK YOU!**



















