



# Using bacteria starters to develop fermented innovative plant-based dairy analogs

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# ➤ Using bacterial starters to develop fermented innovative plant-based dairy analogs

**Gwénaél Jan and Valérie Gagnaire**

INRAE Institut Agro, Science et Technologie du lait et de l'Œuf,  
Rennes, France

# ➤ What are fermented products for us ?

- An ancient way to preserve various raw materials: meat, fish, milk, fruit, plant...
  - Technology driven by cultures and by traditions worldwide
    - A matter of discovery
      - A source of innovation



# > Three main types of fermentation exist...

Sucrose

Fructose, Stachyose

Lactose => Glucose => pyruvate => **lactic acid**

Acetic

Lactic

Propionic

Sucrose => ... => **Ethanol** => **acetic acid**



Sucrose => Glucose => pyruvate => **Ethanol** + **CO<sub>2</sub>**

alcoholic



Malolactic

**Maltose** => Glucose => Pyruvate => **Ethanol** + **CO<sub>2</sub>**

Valence (2019)

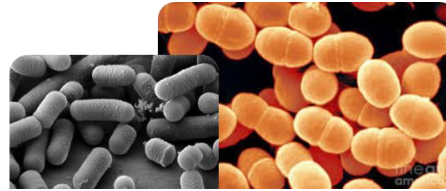
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## > ... using different microorganisms

Lactic

Propionic

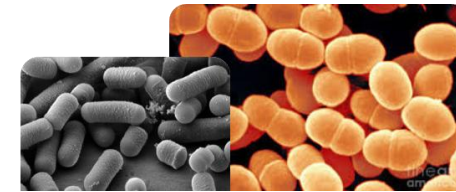


Bacteria

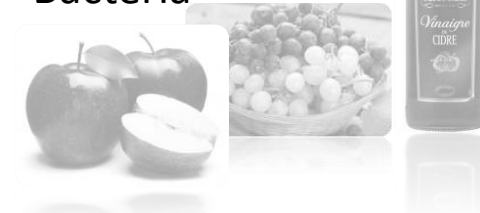
*Lactococcus lactis*  
*Streptococcus thermophilus*  
*Lactiplantibacillus plantarum*  
*Lactobacillus delbrueckii*  
*Propionibacterium freudenreichii*

Acetic

*Acetobacter aceti*



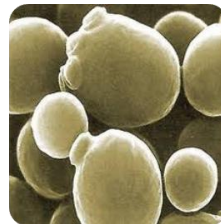
Bacteria



*Saccharomyces cerevisiae*

Alcoholic

Malolactic



Yeast



*Oenococcus oenos*

Bacteria

Valence (2019)

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# ➤ An ancient way to preserve milk as an example

- First archaeological evidences :

- 8 -10 000 years ...

LETTER

doi:10.1038/nature11698

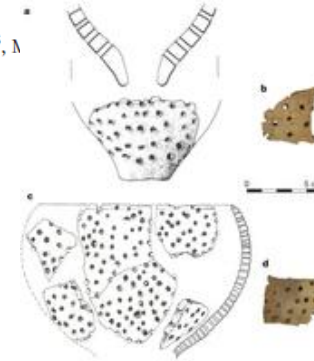
## Earliest evidence for cheese making in the sixth millennium BC in northern Europe

Mélanie Salque<sup>1</sup>, Peter I. Bogucki<sup>2</sup>, Joanna Pyzel<sup>3</sup>, Iwona Sobkowiak-Tabaka<sup>4</sup>, Ryszard Grygiel<sup>5</sup>, A & Richard P. Evershed<sup>1</sup>



(2013), Nature, 493(7433), 522–525.

<https://doi.org/10.1038/nature11698>



- Also described by Aristotle (384 - 322 BC)

**Ex: Kykeon** (Gr. “stir, mix”), Greek beverage made from wine and grated cheese considered as a “magical” medicinal beverage



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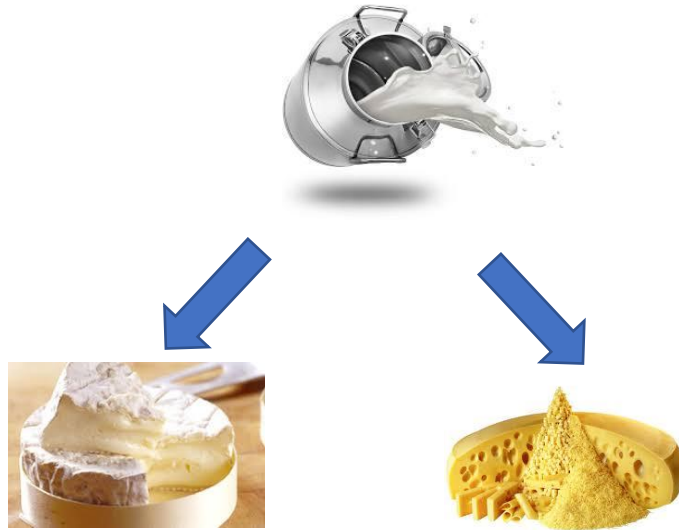
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## ➤ Originally, an empirical selection

Based on environment and technology



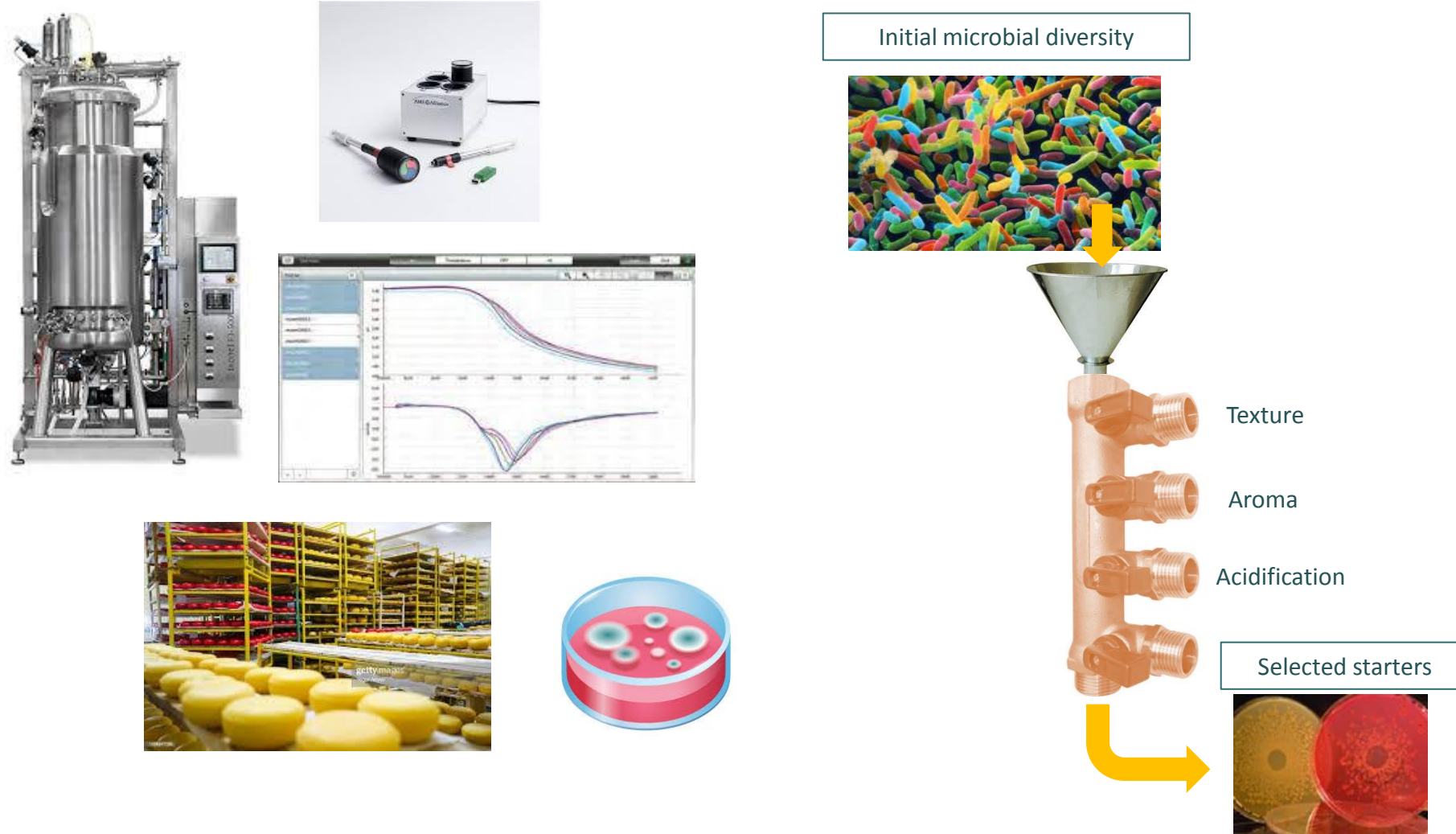
The technology drives the characteristics and microbiota of the products

How were these microorganisms selected?



# ➤ Selection of starter strains

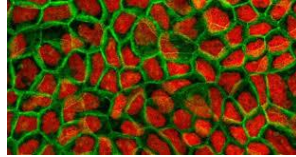
Based on technological performance



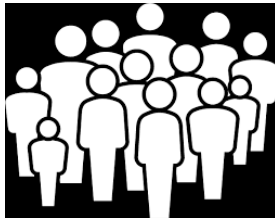


# ➤ Other microorganisms are selected as probiotics

Based on *in vitro*, *in vivo* and clinical screening



IBS, IBD, atopy,  
intolerance ...



Initial microbial diversity



Immunomodulation

Digestion

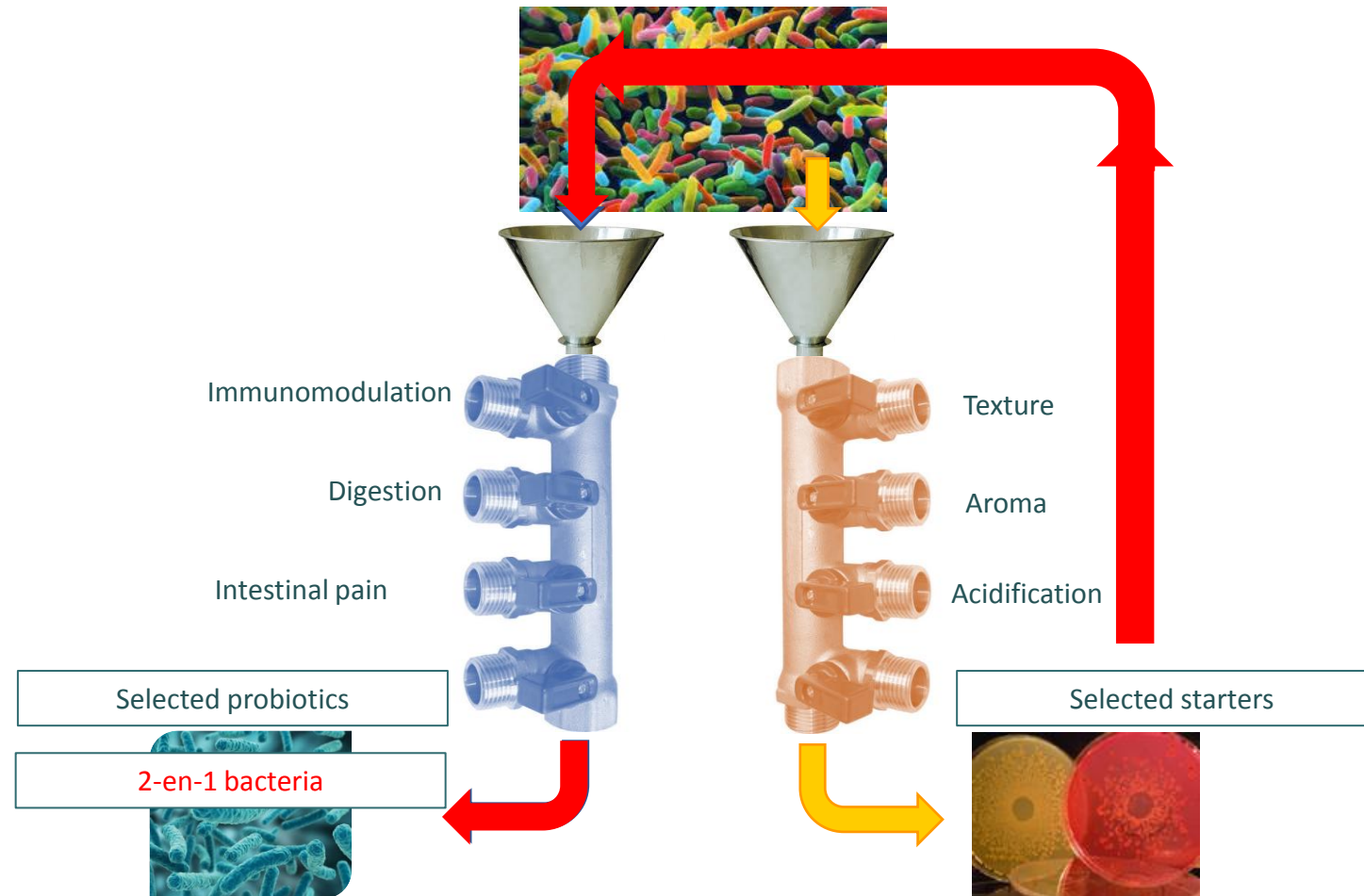
Intestinal pain

Selected probiotics



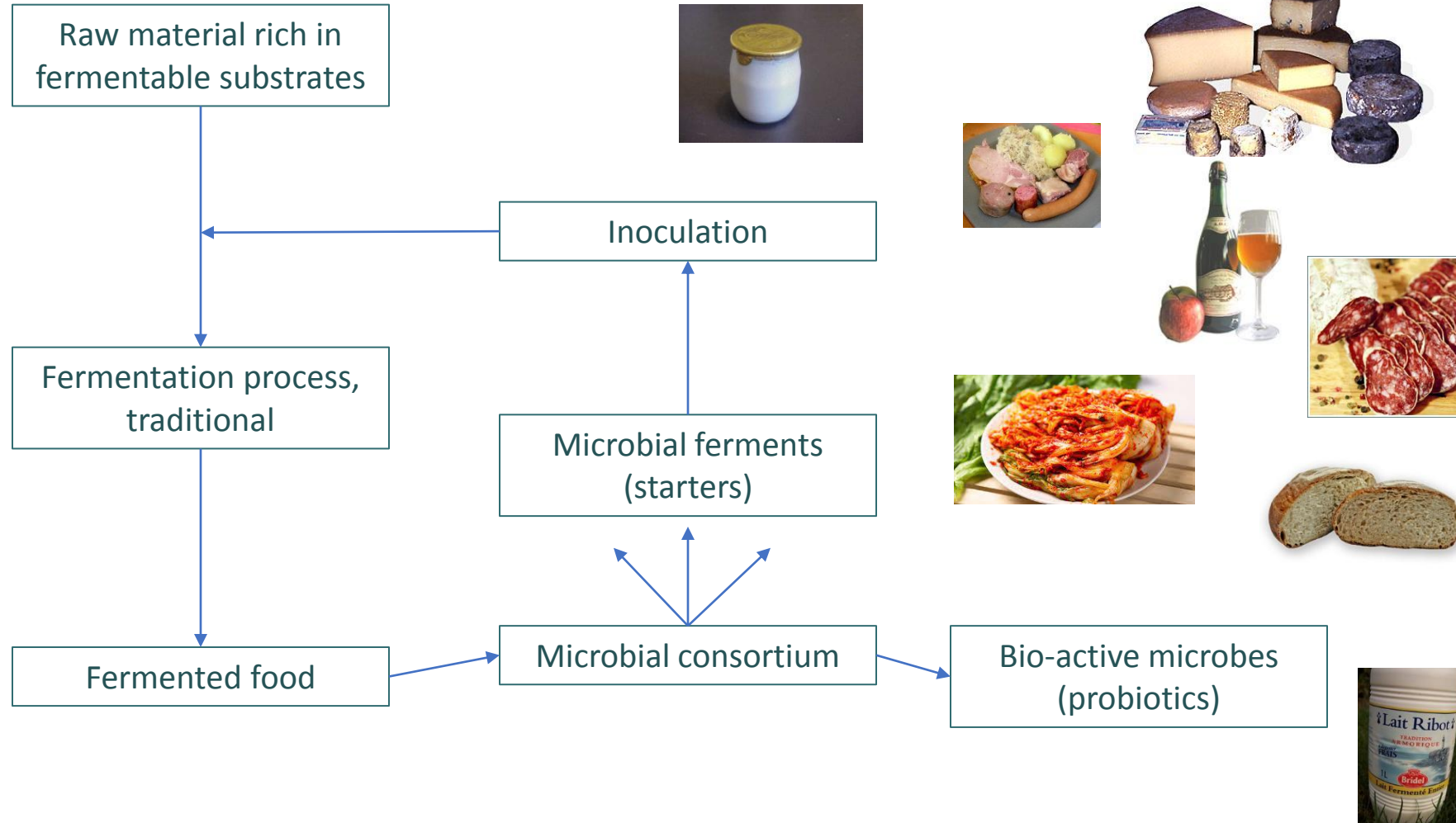
## ➤ We now select “2-in-1” strains

With both technological and probiotic potential



# ➤ But how can fermented foods be a source of probiotics?

Look at how food fermentation works



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# > Yogurt, the best known probiotic product

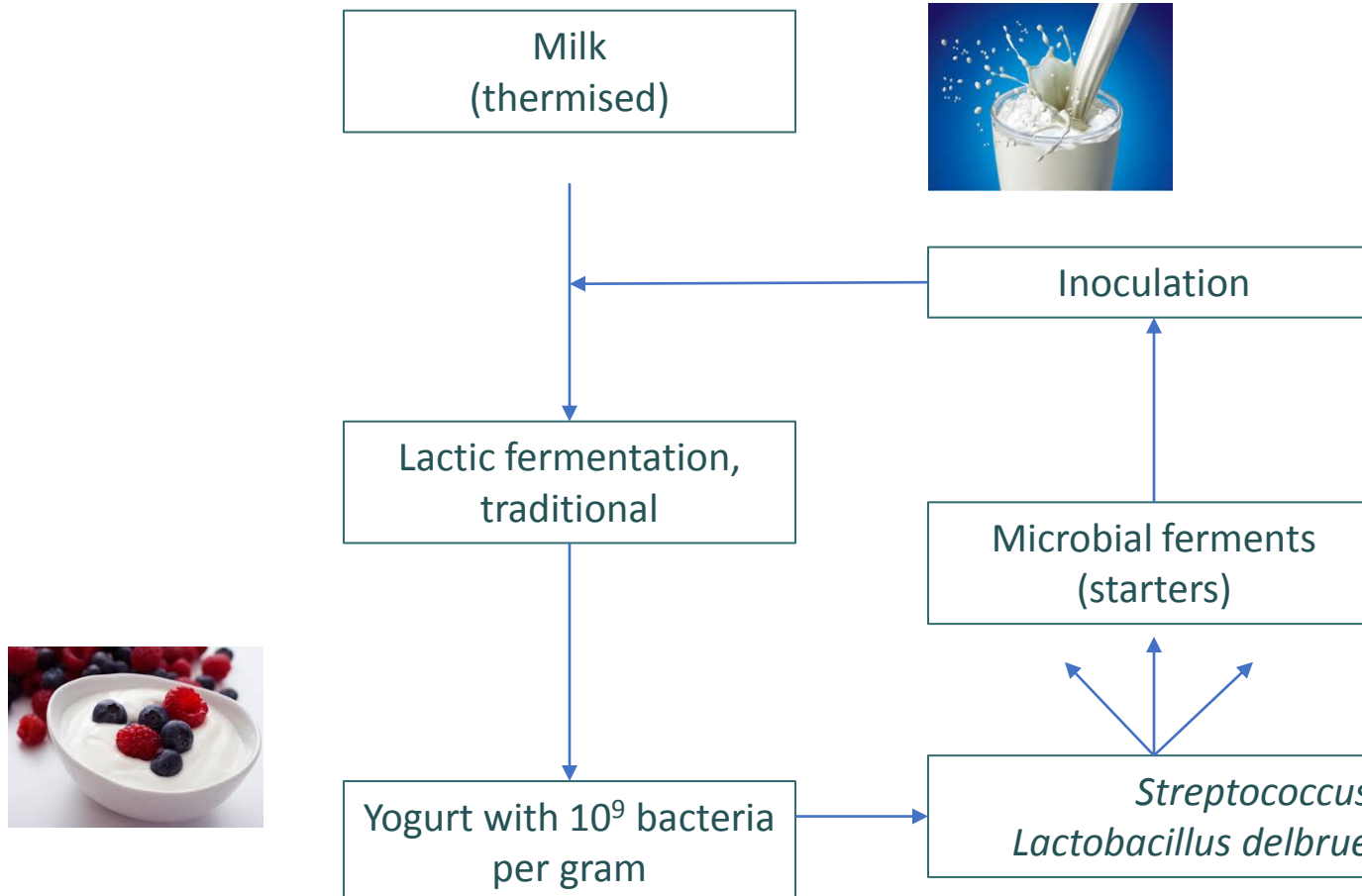
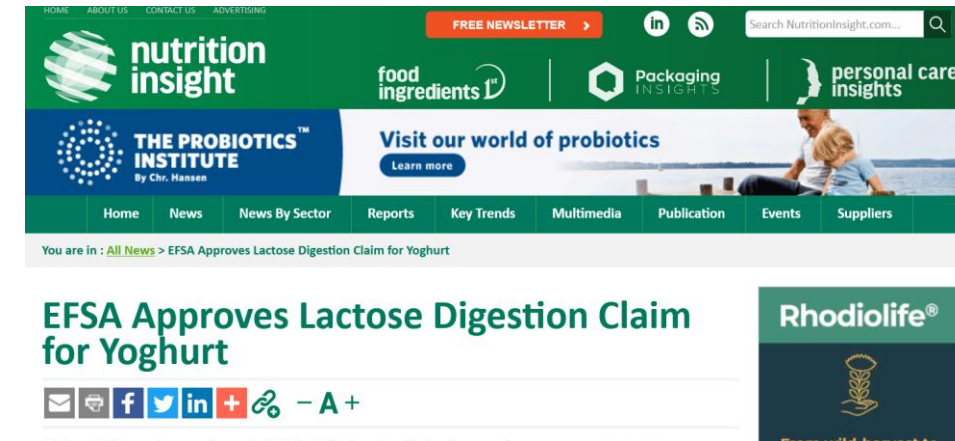
## SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to Article 13(1) of Regulation (EC) No 1924/2006<sup>1</sup>

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)<sup>2,3</sup>

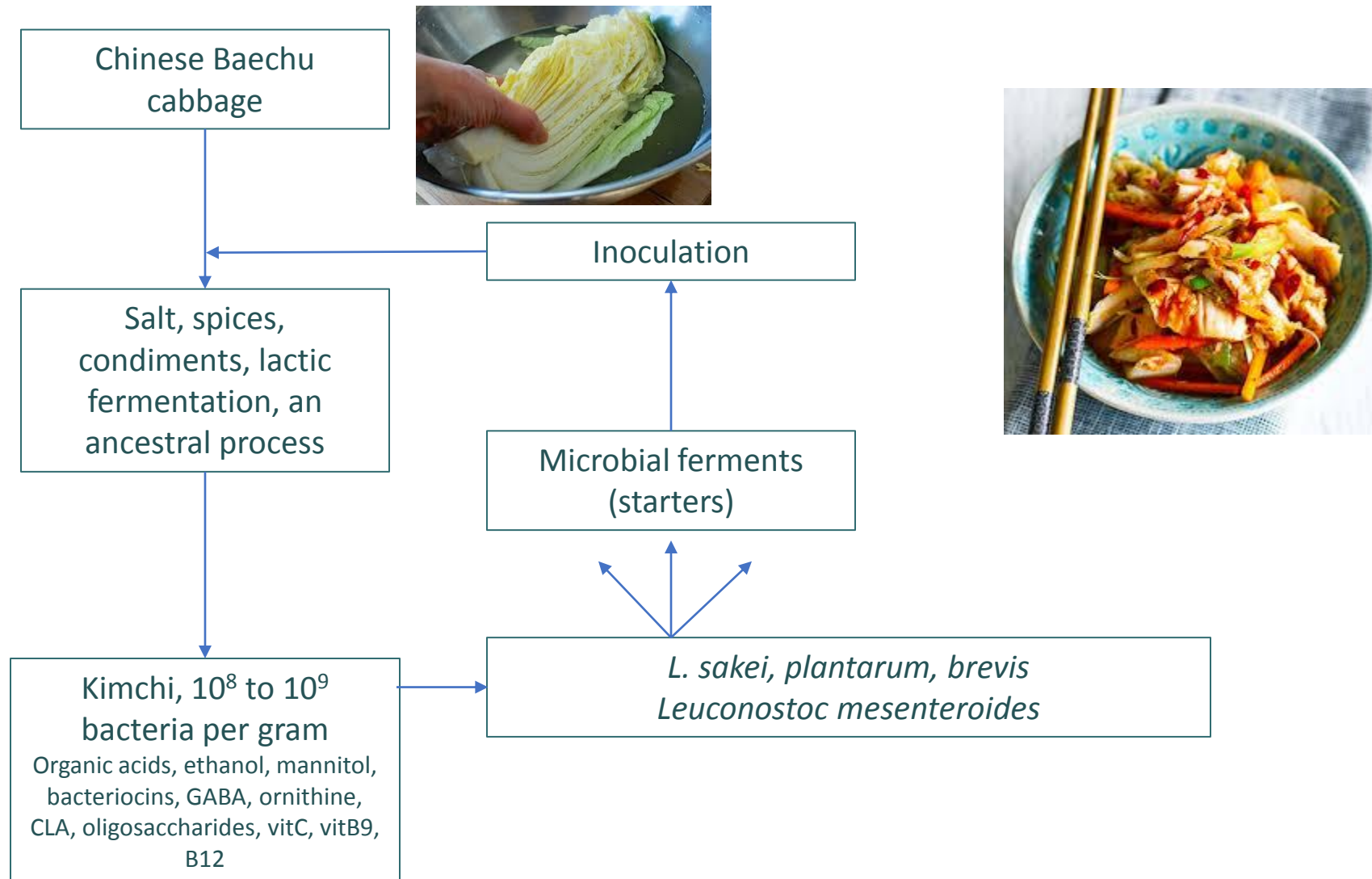
European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 12 January 2011, replaces the earlier version published on 19 October 2010<sup>4</sup>.





## ➤ kimchi, a traditional probiotic vegetable fermented food





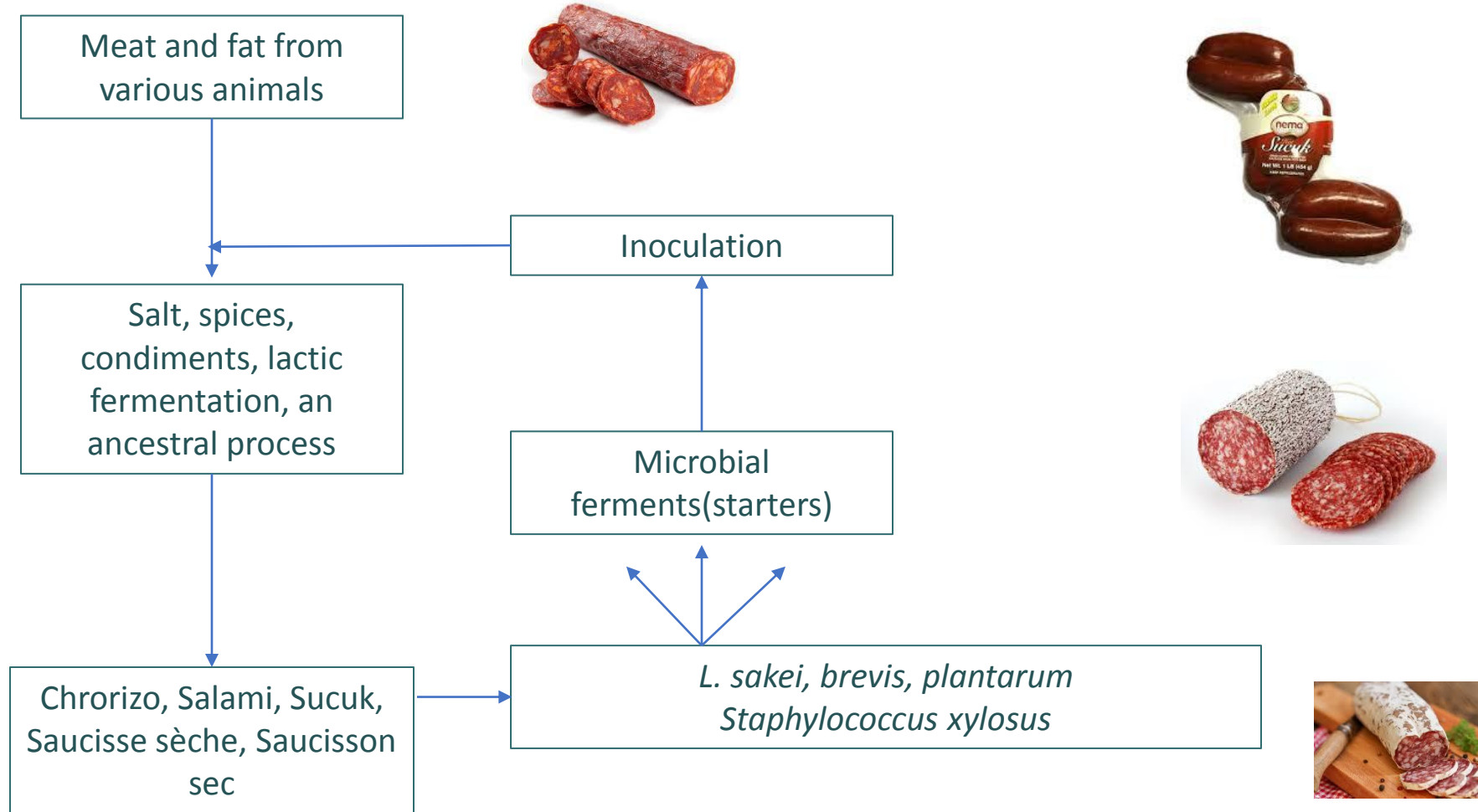
## ➤ *Lactiplantibacillus plantarum*, a probiotic bacterium? At least strain 299v...



- Consumption of *L. plantarum*
- ↓ severity of visceral pain
  - ↓ defecation frequency
  - ↓ bloating



## ➤ Fermented sausages



## ➤ *Latilactobacillus sakei*, a probiotic bacterium?



Annals of Allergy, Asthma & Immunology

Volume 104, Issue 4, April 2010, Pages 343-348



Original article

Intervention

### Effect of *Lactobacillus sakei* supplementation in children with atopic eczema–dermatitis syndrome

Sung-Il Woo MD \*, Ji-Yoon Kim MD \*, Yong-Ju Lee MD \*, Nam-Shik Kim PhD†, Youn-Soo Hahn MD, PhD \* ʘ ʘ

#### CONCLUSIONS:

Supplementation of *L. sakei* in children with AEDS was associated with a substantial clinical improvement and a significant decrease in chemokine levels, reflecting the severity of AEDS.



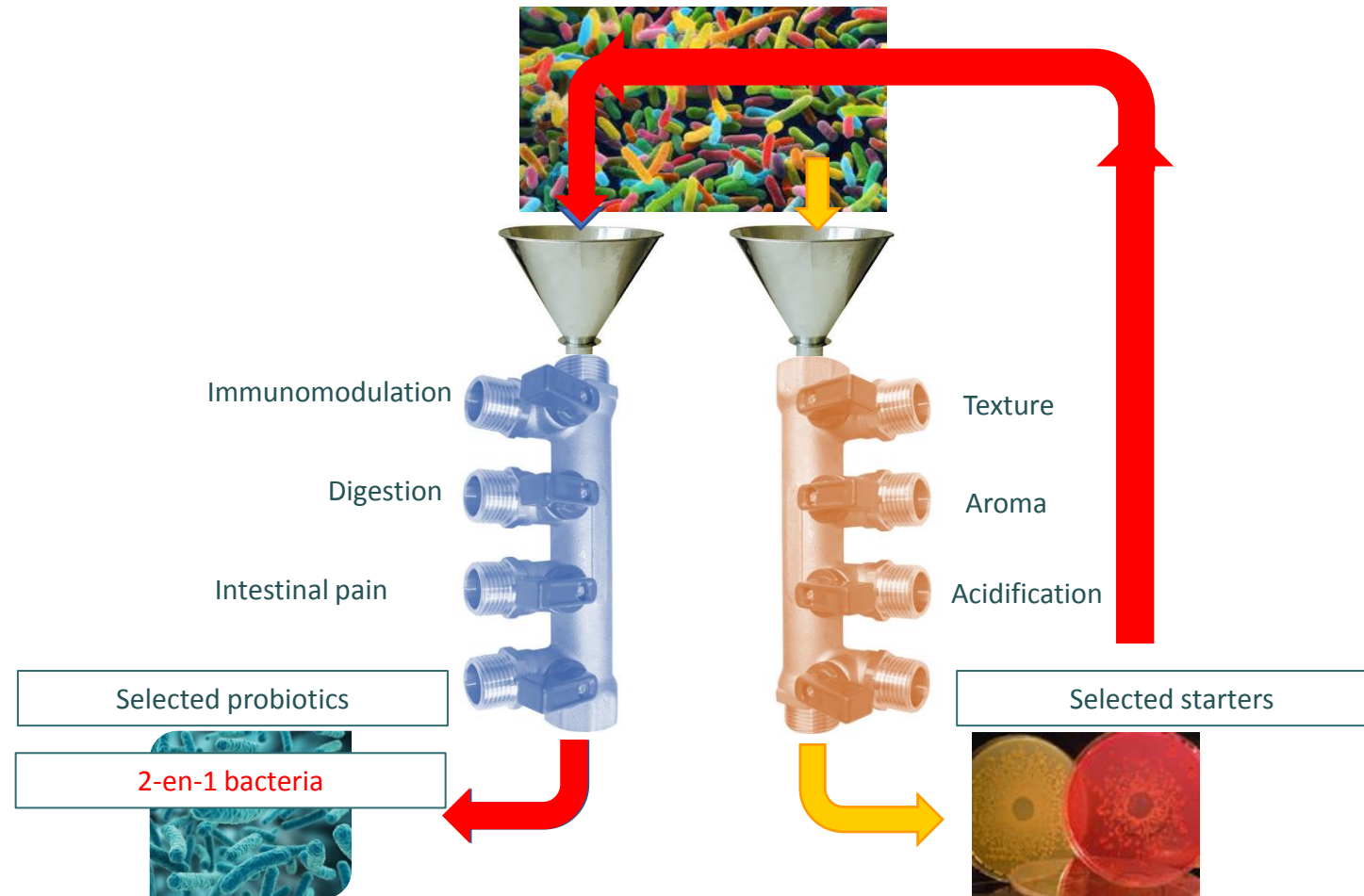
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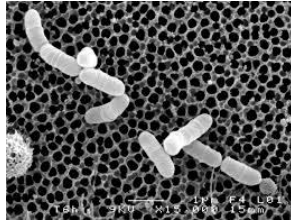
## ➤ Back to selection of “2-in-1” strains

With both technological and probiotic potential



# ➤ As an example....

## Starters of Emmental cheese

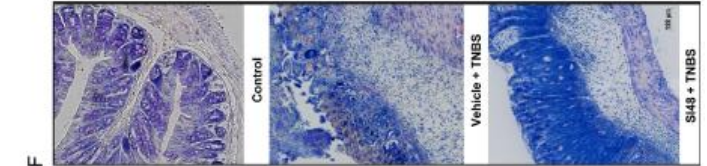


APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2010, p. 8259–8264  
0099-2240/10/\$12.00 doi:10.1128/AEM.01976-10  
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

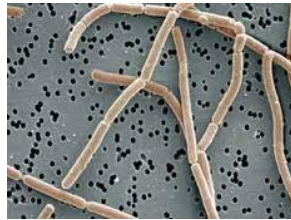
Vol. 76, No. 24

### Promising Immunomodulatory Effects of Selected Strains of Dairy Propionibacteria as Evidenced *In Vitro* and *In Vivo*<sup>†</sup>

Benoit Foligné,<sup>1,2,3,4</sup> Stéphanie-Marie Deutsch,<sup>5,6</sup> Jérôme Breton,<sup>1,2,3,4</sup> Fabien J. Cousin,<sup>5,6,7</sup> Joëlle Dewulf,<sup>1,2,3,4</sup> Michel Samson,<sup>8</sup> Bruno Pot,<sup>1,2,3,4</sup> and Gwénaél Jan<sup>5,6\*</sup>



### *Propionibacterium freudenreichii*

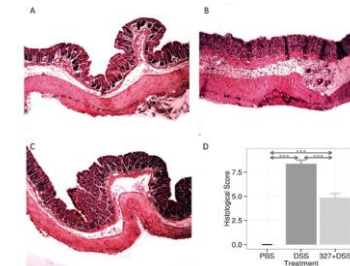


OPEN ACCESS Freely available online

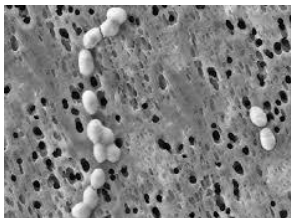
PLOS ONE

### Local and Systemic Immune Mechanisms Underlying the Anti-Colitis Effects of the Dairy Bacterium *Lactobacillus delbrueckii*

Clarissa Santos Rocha<sup>1,3,4</sup>, Ana Cristina Gomes-Santos<sup>2</sup>, Thais Garcias Moreira<sup>2</sup>, Marcela de Azevedo<sup>1</sup>, Tessalia Diniz Luerce<sup>1</sup>, Mahendra Mariadassou<sup>5</sup>, Ana Paula Longaray Delamare<sup>6</sup>, Philippe Langella<sup>3,4</sup>, Emmanuelle Maguin<sup>3,4</sup>, Vasco Azevedo<sup>1</sup>, Ana Maria Caetano de Faria<sup>2</sup>, Anderson Miyoshi<sup>1\*</sup>, Maarten van de Guchte<sup>3,4,5</sup>



### *Lactobacillus delbrueckii*



frontiers  
in Physiology

ORIGINAL RESEARCH  
published: 24 July 2018  
doi: 10.3389/fphys.2018.00980



OPEN ACCESS

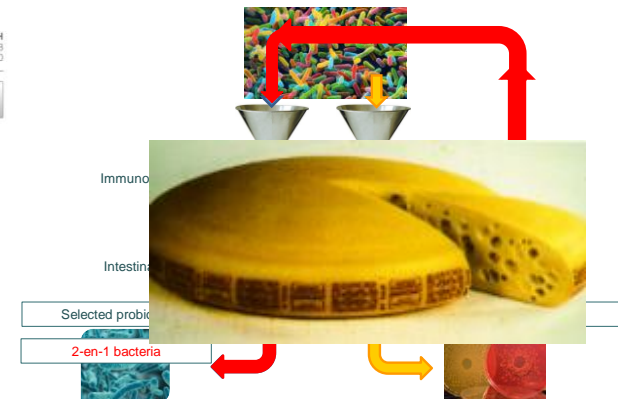
Edited by:  
Stephen J. Pandolfi,  
Cedars-Sinai Medical Center,  
United States

Reviewed by:  
Philipp Hartmann,  
University of California, San Diego,  
United States  
Mauricio J. Galar

### Characterization of Mucus-Related Properties of *Streptococcus thermophilus*: From Adhesion to Induction

Neiké Fernandez<sup>1†</sup>, Laura Wrzosek<sup>1†</sup>, Joanna M. Radziwill-Bienkowska<sup>2</sup>, Belinda Ringot-Destrez<sup>1,4,5</sup>, Marie-Pierre Duviau<sup>4</sup>, Marie-Louise Noordine<sup>1</sup>, Valérie Laroute<sup>6</sup>, Véronique Robert<sup>1</sup>, Claire Cherbuy<sup>1</sup>, Marie-Line Davaeran-Mingot<sup>4</sup>, Muriel Coccagn-Bousquet<sup>4</sup>, Renaud Léonard<sup>1,4,5</sup>, Catherine Robbe-Masselot<sup>1,4,5</sup>, Françoise Rul<sup>1</sup>, Eric Ogier-Denis<sup>1,4,5</sup>, Muriel Thomas<sup>1,4</sup> and Muriel Mercier-Bonin<sup>1†</sup>

### *Streptococcus thermophilus*



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# > In DSS-induced colitis

Emmental cheese with  
*P. freudenreichii*  
*S. thermophilus*  
*L. delbrueckii*

C57BL6  
8<sup>th</sup> week age



1<sup>st</sup> Day



5<sup>th</sup> Day

12<sup>th</sup> Day

Intragastric gavage

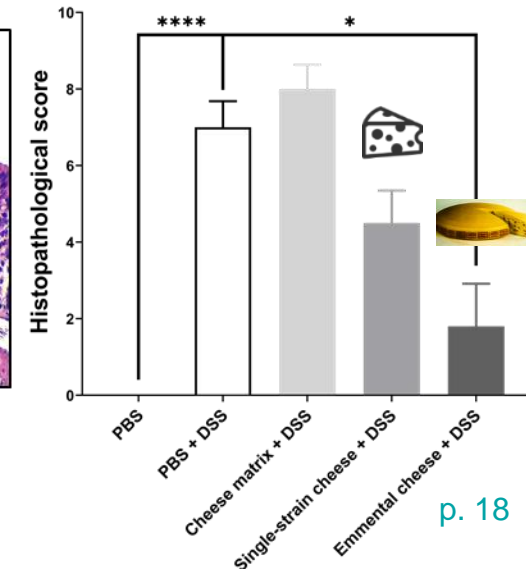
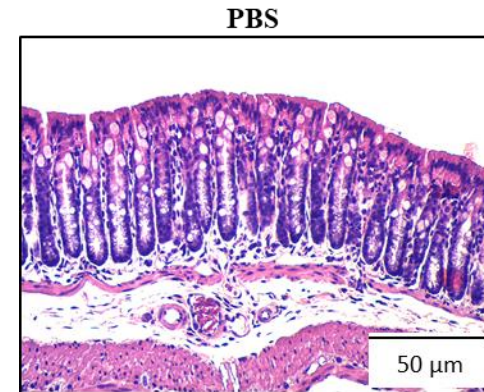
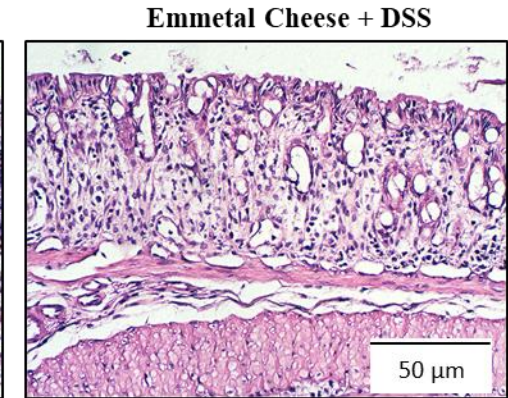
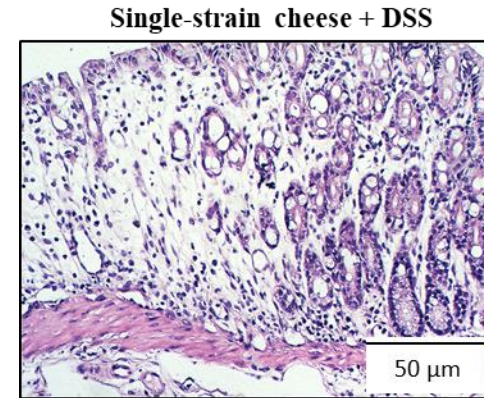
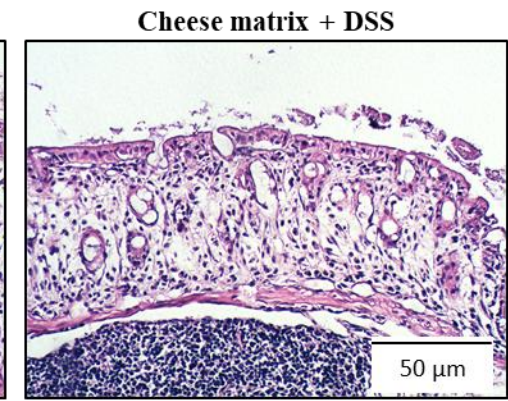
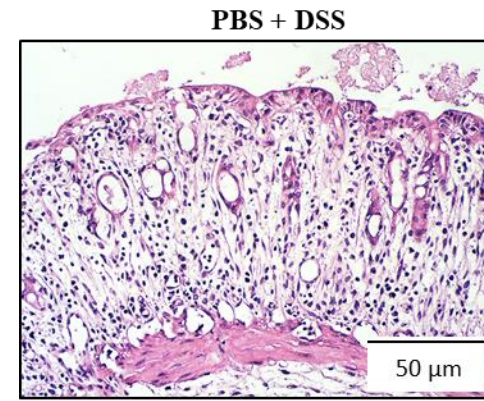
Gavage 500  $\mu$ L  
(400 mg cheese in  
500  $\mu$ L PBS pH 7.4 )

3% (w/v) DSS solution  
(36–50 25 kDa)

Colitis induction

Beginning

Euthanasia



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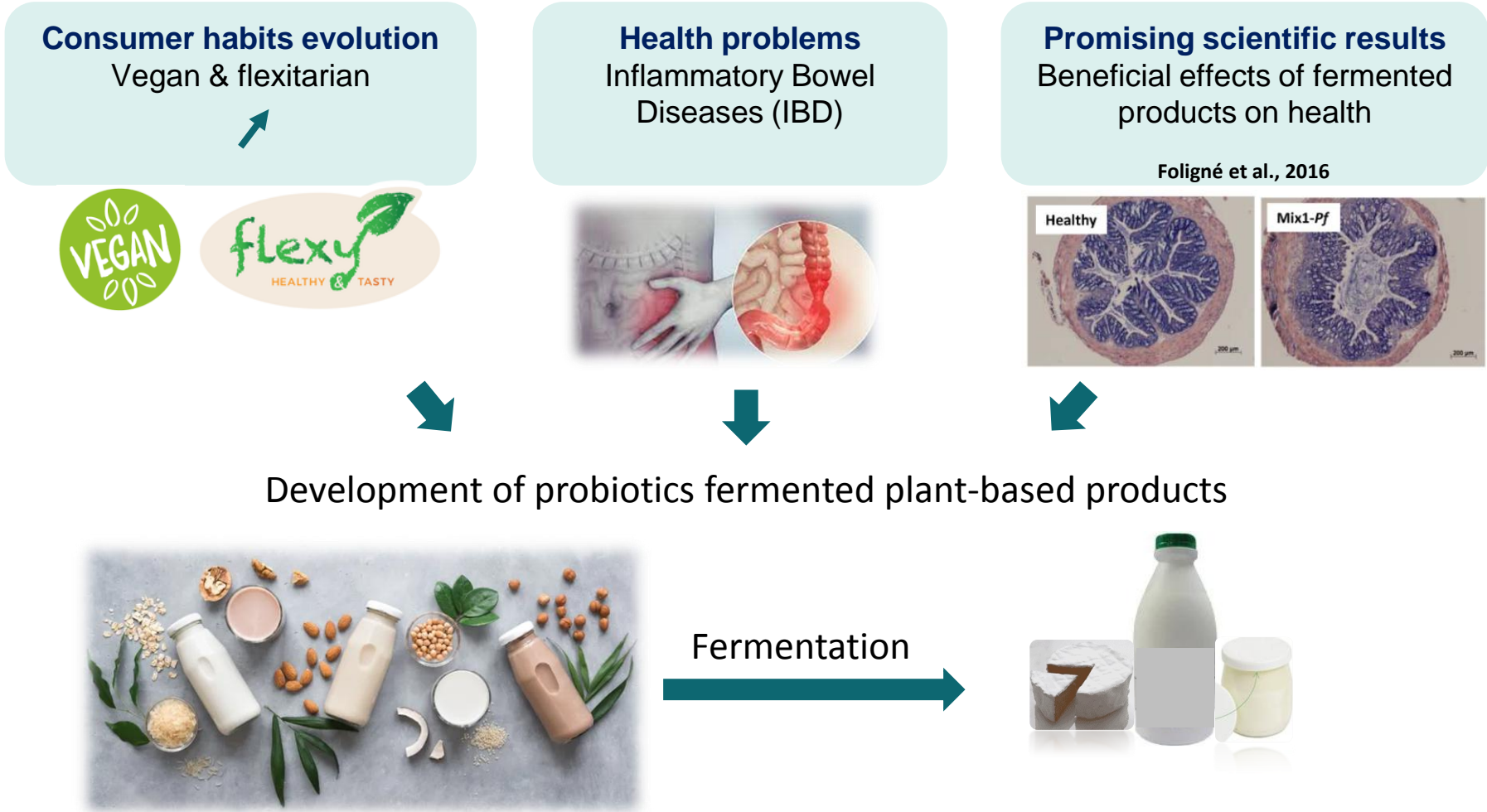
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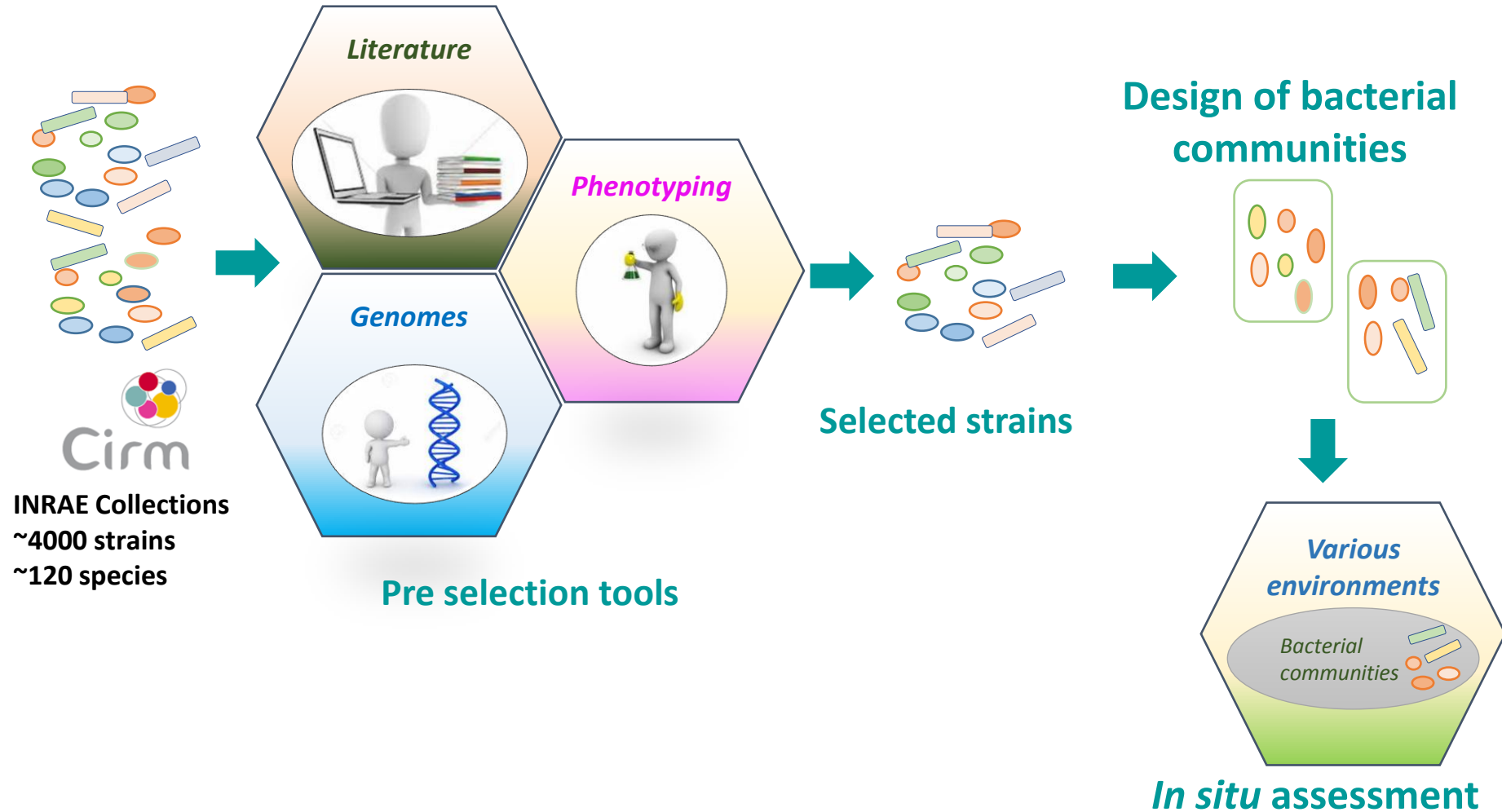
- But this is a fermented dairy product.....
- We now shift to fermented vegetable products
  - What do we expect from our « 2-in-1 » bacteria?
- Degrade plant sugars:  $\alpha$ -galactosidase rather than  $\beta$ -galactosidase?
- Degrade plant proteins: enhance bio-availability of N, reduce allergens?
- Reduce inflammation, prevent immune disorders?
- ...



# > Growing demand for plant-based fermented products...



## ➤ New tools to select bacteria of interest





## ➤ New fermented products mixing milk and legumes

- Incorporation of vegetable proteins, and of bacteria able to process them, into the cheese-making process, leads to innovative cheeses with a reduced ecological impact.
  - We developed an original approach of *in silico* and *in vitro* screening, and clustering of lactic acid bacteria strains to design communities that have complementary metabolism



# Function-Driven Design of Lactic Acid Bacteria Co-cultures to Produce New Fermented Food Associating Milk and Lupin

Fanny Canon<sup>1</sup>, Mahendra Mariadassou<sup>2</sup>, Marie-Bernadette Maillard<sup>1</sup>, H  l  ne Falentin<sup>1</sup>, Sandrine Parayre<sup>1</sup>, Marie-No  lle Madec<sup>1</sup>, Florence Valence<sup>1</sup>, Gw  na  le Henry<sup>1</sup>, Val  rie Laroute<sup>3</sup>, Marie-Line Daveran-Mingot<sup>3</sup>, Muriel Coca  gn-Bousquet<sup>3</sup>, Anne Thierry<sup>1†</sup> et Val  rie Gagnaire<sup>1\*†</sup>









<sup>1</sup> INRAE, Institut Agro, STLO, Rennes, France, <sup>2</sup> INRAE, UR1404 MalAGE, Jouy-en-Josas, France, <sup>3</sup> Université de Toulouse, CNRS, INRAE, INSA, TBI, Toulouse, France





# ➤ Design of bacterial communities able to ferment a new food combining milk and legumes

Some of the characteristics obtained after the fermentation of milk-legumes mixes:

-  stables 
- Legume oligosaccharides  ➡ After fermentation 
- Hexanal  ➡ After fermentation 
- Aroma compound variably produced according to the communities used, sensorial impact 
- « Predigestion » of the proteins variable according to the communities used compared to the unfermented mixes : acceleration of the digestion during gastric phase under *in vitro* conditions 

## First rules of lactic acid bacteria (LAB) association

- **Cooperation** (commensalism and even mutualism) : proteolytic products as a way to promote interactions between LAB strains

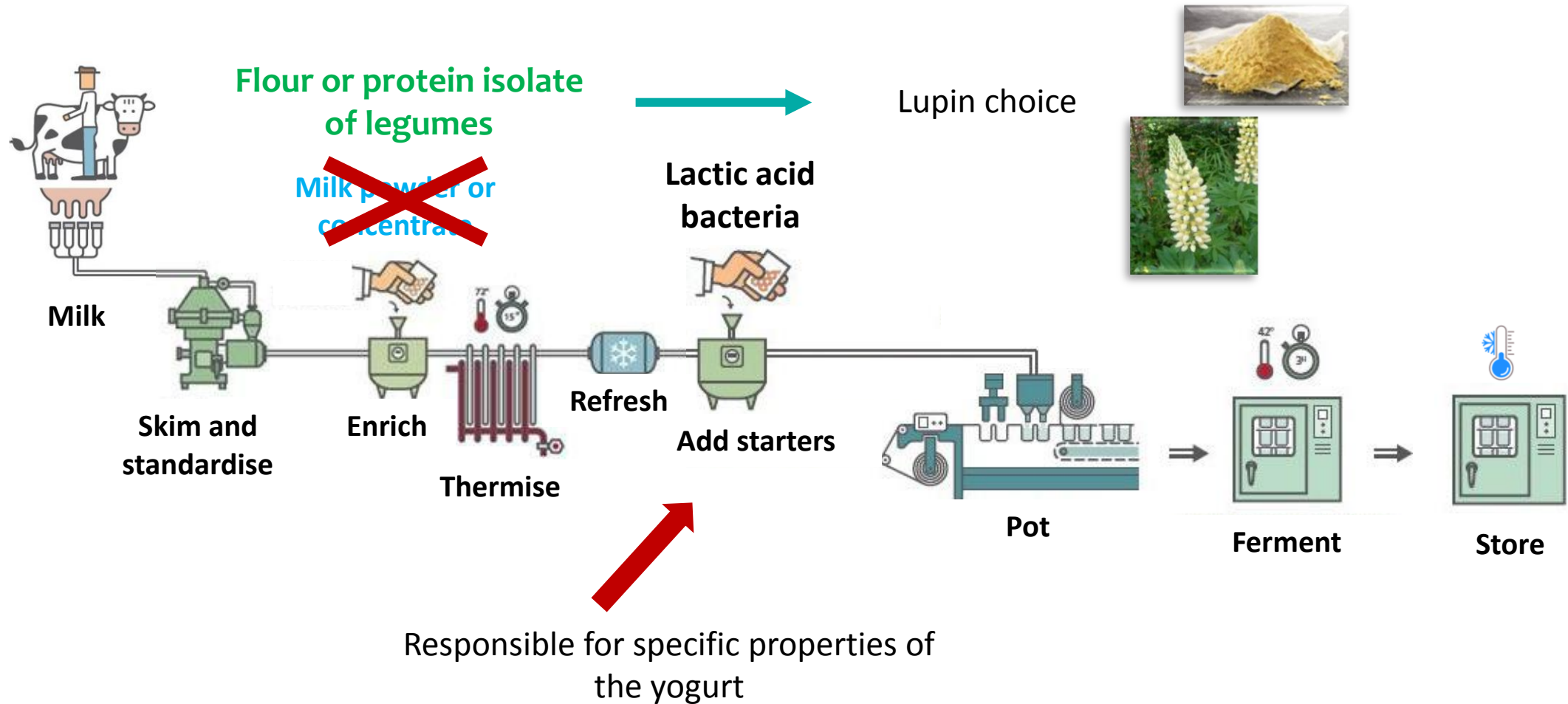
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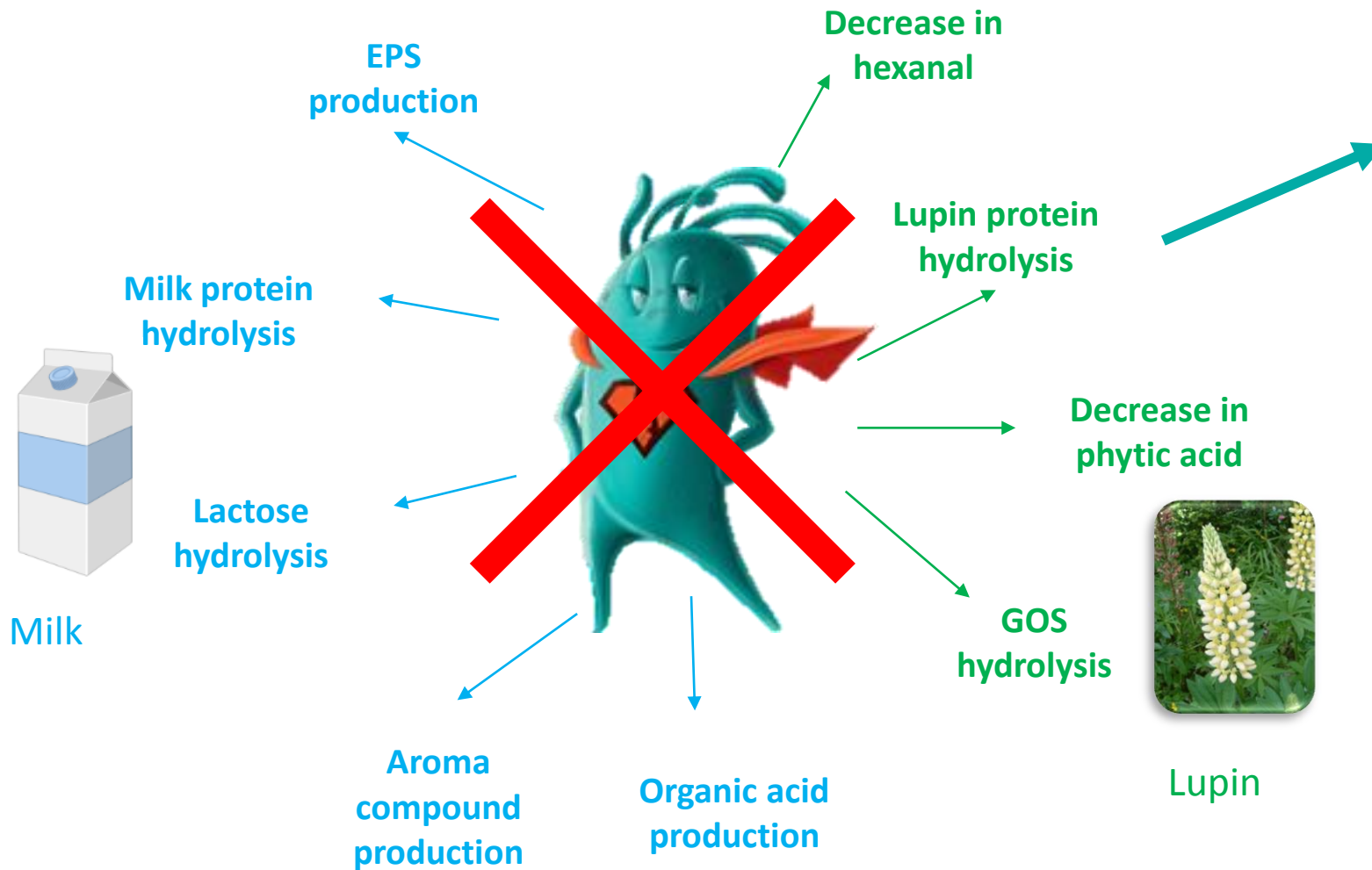
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# ➤ Scheme of yogurt and fermented milk production

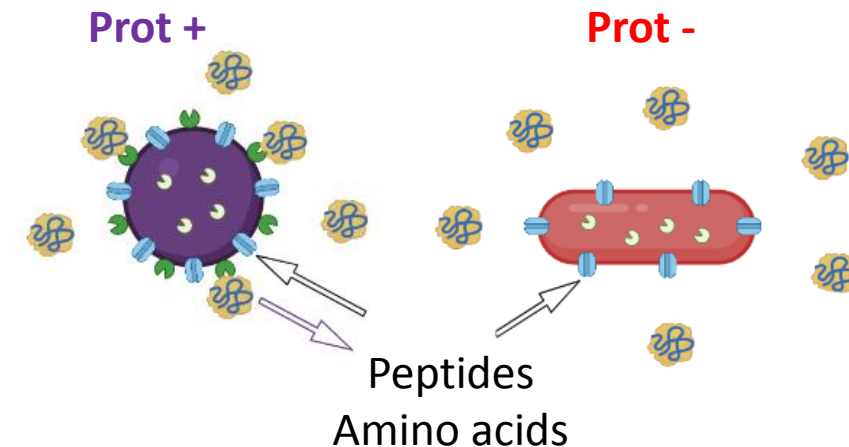


# ➤ Objective : to add up functionalities of the lactic acid bacteria in fermented mixed plant and dairy based “yogurts”



## Association of several strains required

Chosen approach: to favour positive interactions between strains based on their nitrogen metabolism



Canon et al (2021)

# ➤ Experimental design for mixed milk-lupin yogurt manufacture

## Three factors

## Levels

Proteolytic activity

## Responses

5 starter cultures

Monocultures

- *E. faecalis* (F) +++
- *L. lactis* (L) ++
- *L. plantarum* (P) -

Cocultures

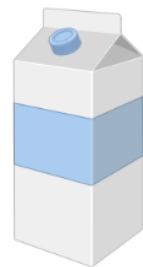
- F x P
- L x P

2 fat types

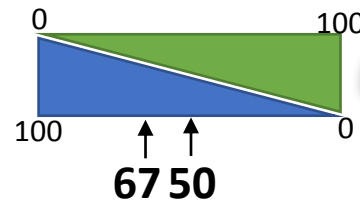
Milk fat  
(1,5 %)

Coconut oil  
(1,5 %)

2 milk/lupin  
protein ratios



Milk



Lupin

Bacterial growth

Acidification

Proteolysis

Volatile compounds and organic acid determination

Physical Properties

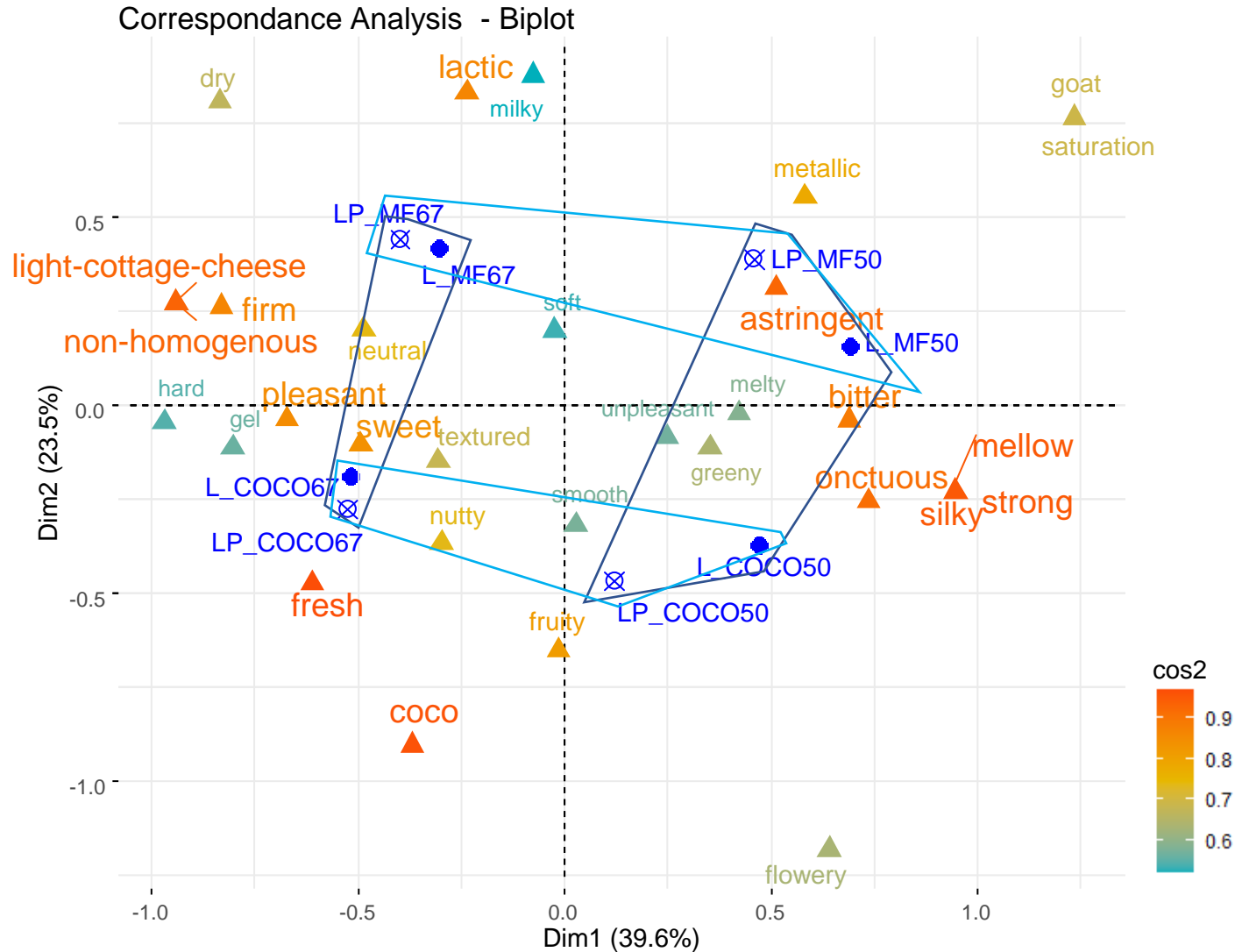
Firmness

Viscosity

Water holding capacity (WHC)

Sensory analyses on L and L x P yogurts

# ➤ Sensory analyses (sorting task) also driven by the composition when L and L x P cultures are used



- **Milk/lupin protein ratio** differentiated on the 1<sup>st</sup> axis
  - ratio 50: unpleasant, bitter and with a mellow texture
  - ratio 67: pleasant, textured (hard gel) and nonhomogeneous
- **Fat type** differentiated on the 2<sup>nd</sup> axis
  - Milk fat: milky, lactic and “goaty”
  - coco as fruity, fresh and nutty

Untrained panellists



## ➤ What about fermented 100% vegetable dairy analogues?



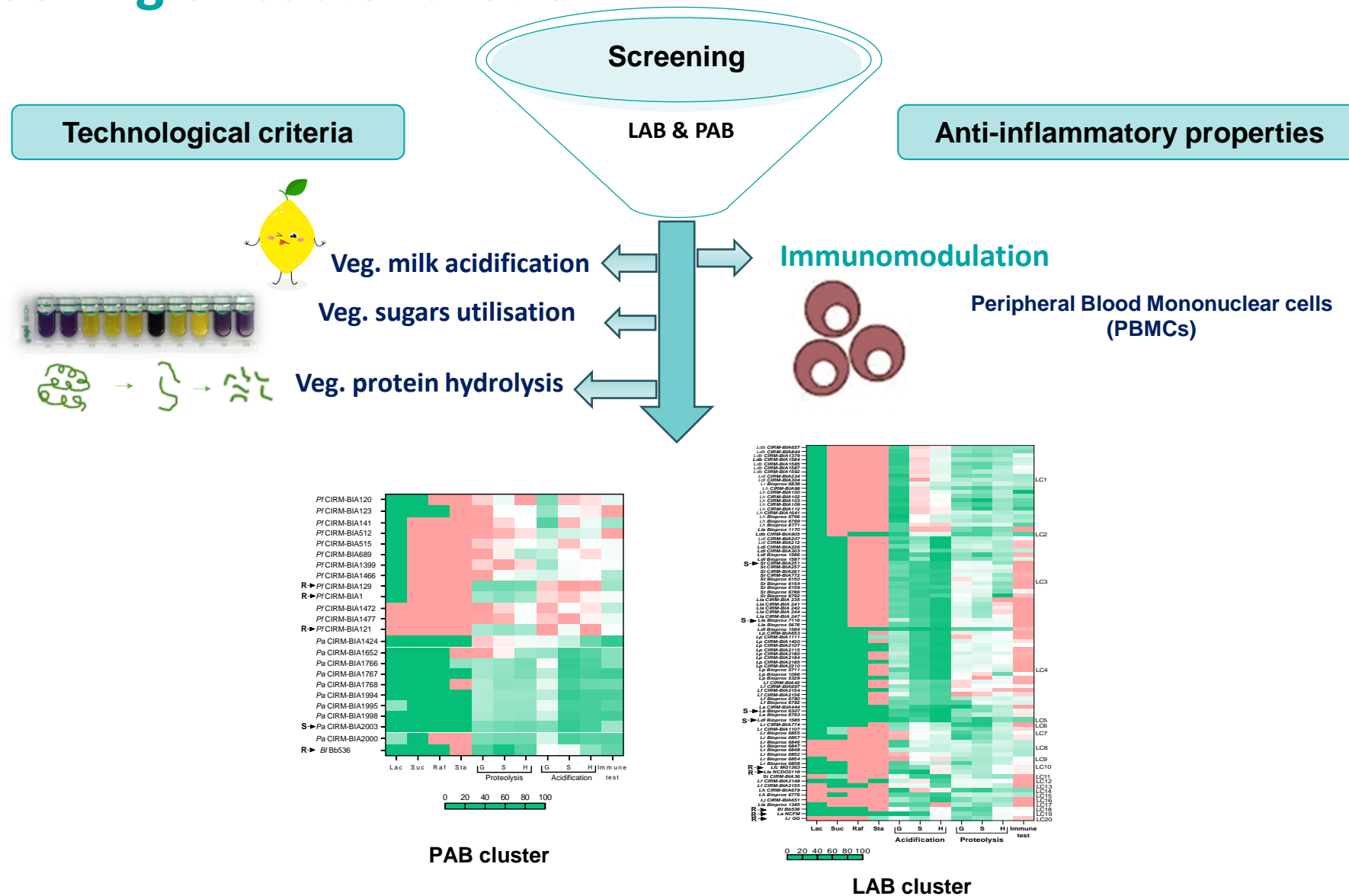
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# ➤ Screening of bacterial strains



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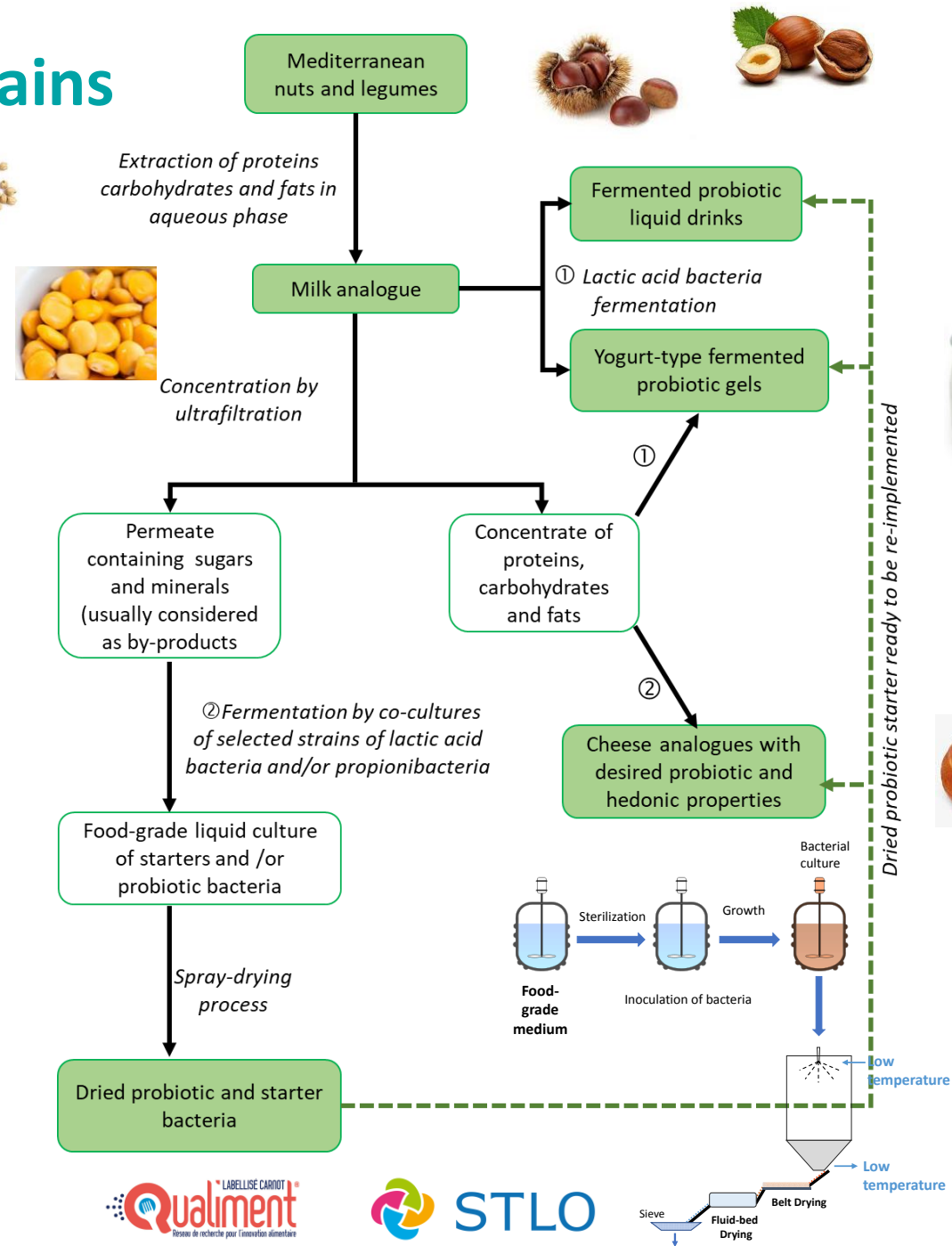
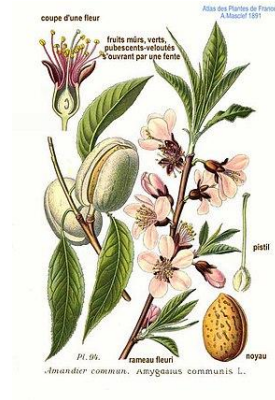
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# ➤ Implementation of these strains

WP2 of Localnutleg project

Thank you for your attention



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