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

Gwénaél Jan, Valérie Gagnaire

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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 + CO₂**

alcoholic



Malolactic

Maltose => Glucose => Pyruvate => **Ethanol + CO₂**

Valence (2019)

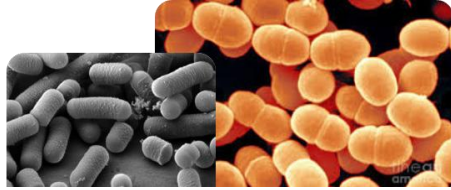
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> ... using different microorganisms

Lactic

Propionic



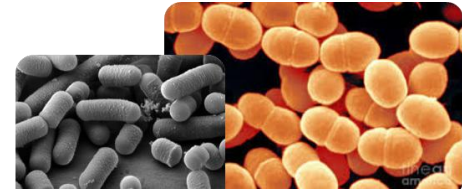
Bacteria

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



Acetic

Acetobacter aceti



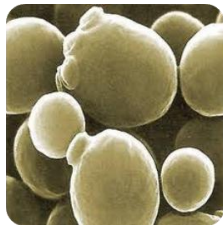
Bacteria



Saccharomyces cerevisiae

Alcoholic

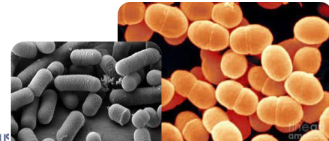
Malolactic



Yeast



Oenococcus oenos



Bacteria

Valence (2019)



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Bacteria

➤ An ancient way to preserve milk as an example

- First archaeological evidences :
 - 8 -10 000 years ...

LETTER

doi:10.1038/nature11698

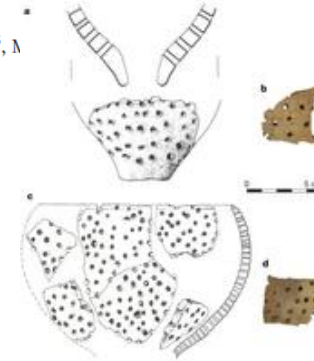
Earliest evidence for cheese making in the sixth millennium BC in northern Europe

Mélanie Salque¹, Peter I. Bogucki², Joanna Pyzel³, Iwona Sobkowiak-Tabaka⁴, Ryszard Grygiel⁵, M & Richard P. Evershed¹



(2013), Nature, 493(7433), 522–525.

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



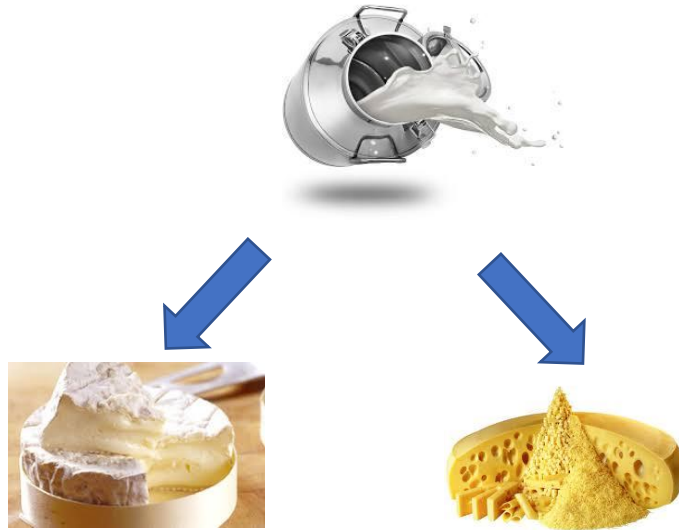
- Also described by Aristotle (384 - 322 BC)

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



➤ Originally, an empirical selection

Based on environment and technology



How were these microorganisms selected?

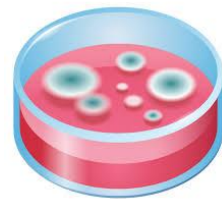
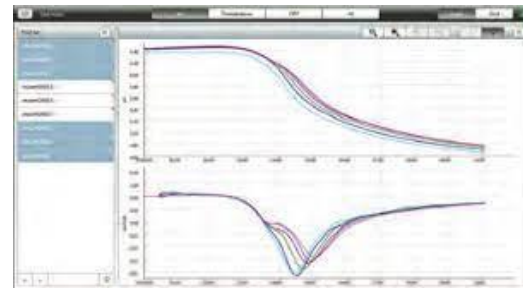


The technology drives the characteristics and microbiota of the products

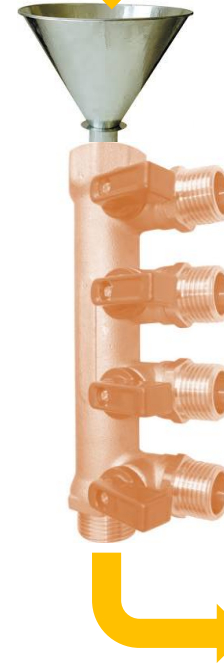


➤ Selection of starter strains

Based on technological performance



Initial microbial diversity



Texture

Aroma

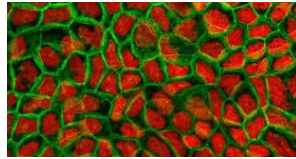
Acidification

Selected starters



➤ Other microorganisms are selected as probiotics

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



IBS, IBD, atopy,
intolerance ...



Initial microbial diversity



Immunomodulation

Digestion

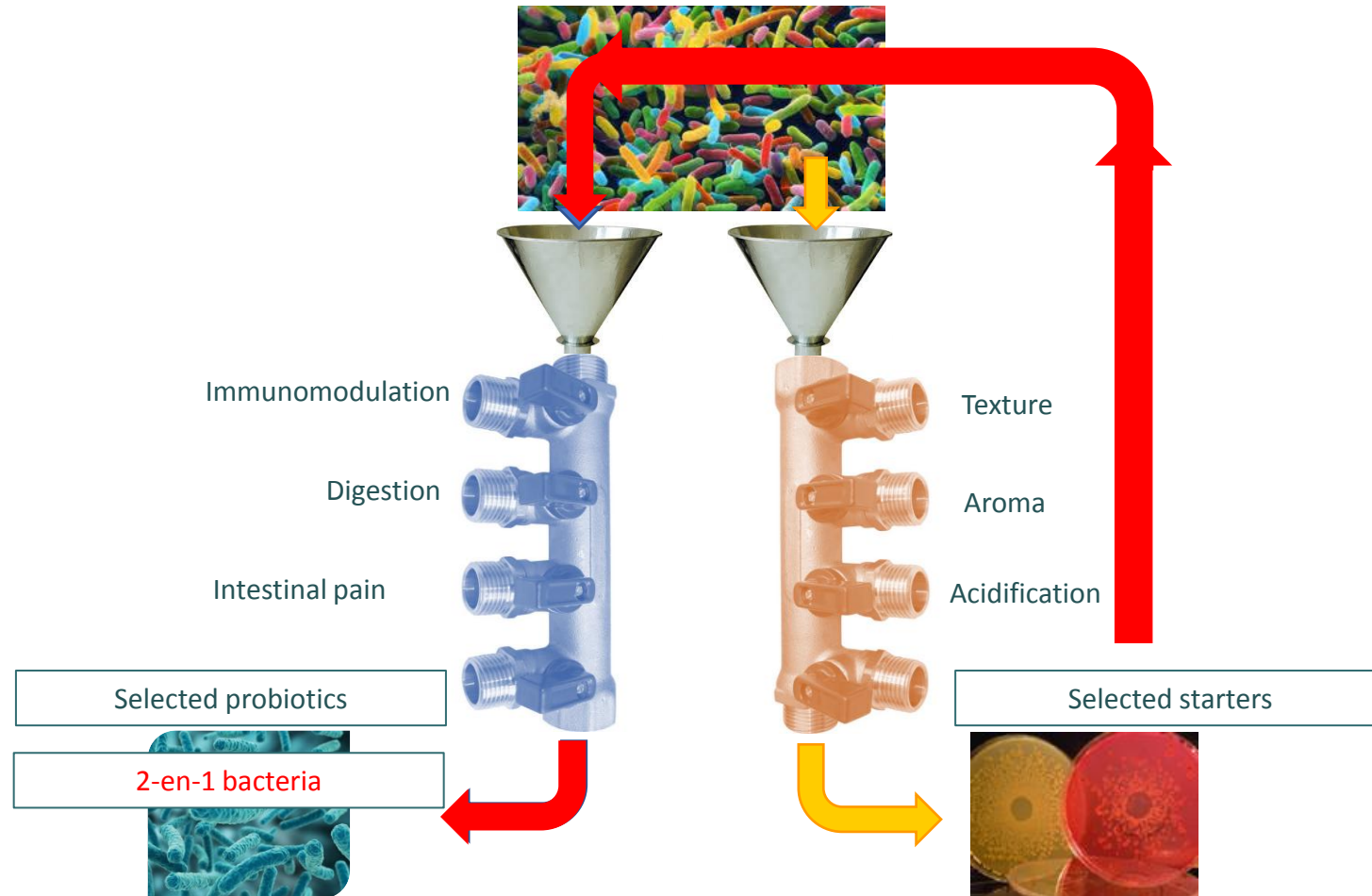
Intestinal pain

Selected probiotics



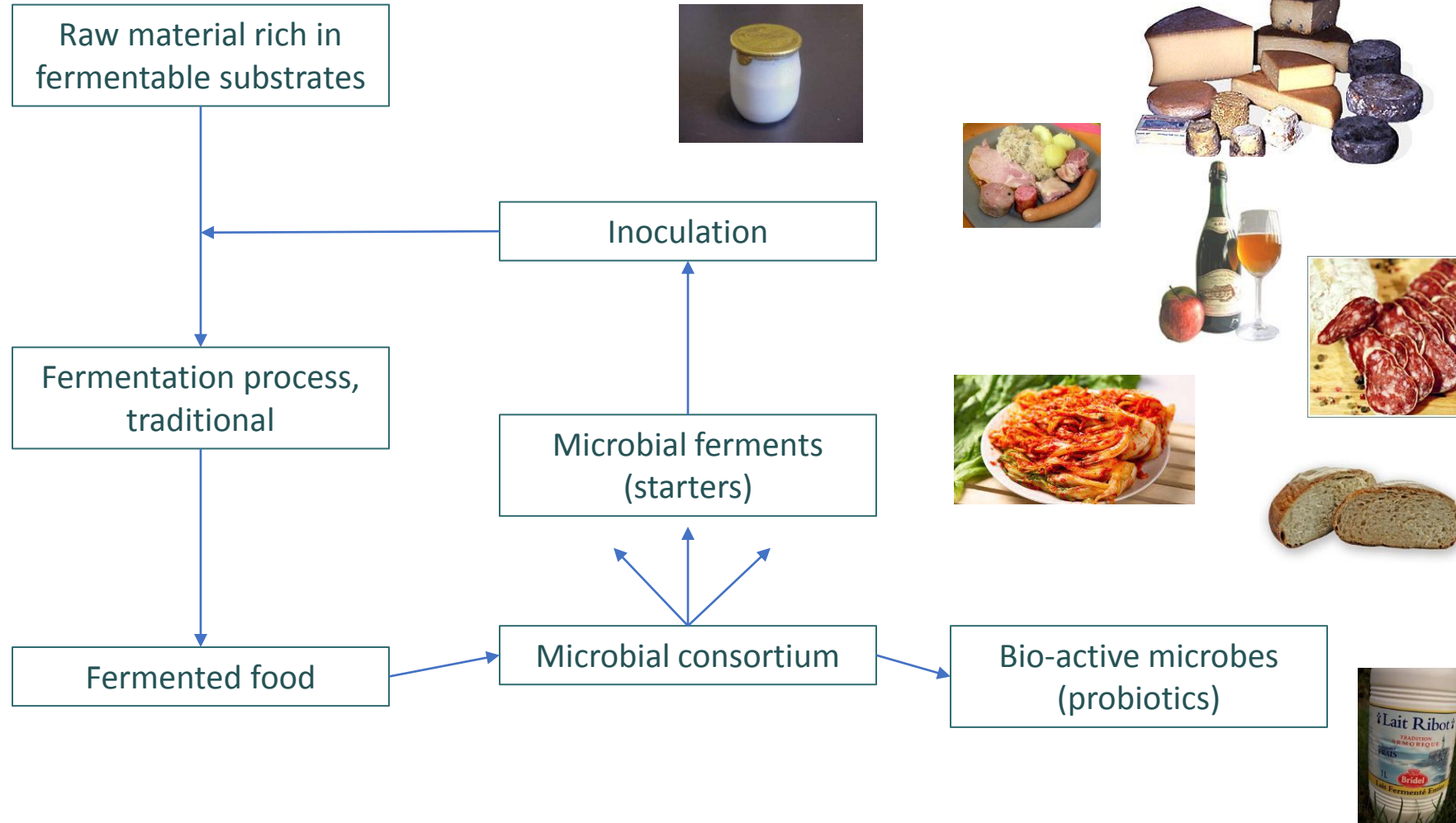
➤ We now select “2-in-1” strains

With both technological and probiotic potential



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

Look at how food fermentation works



➤ 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 1924/2006¹

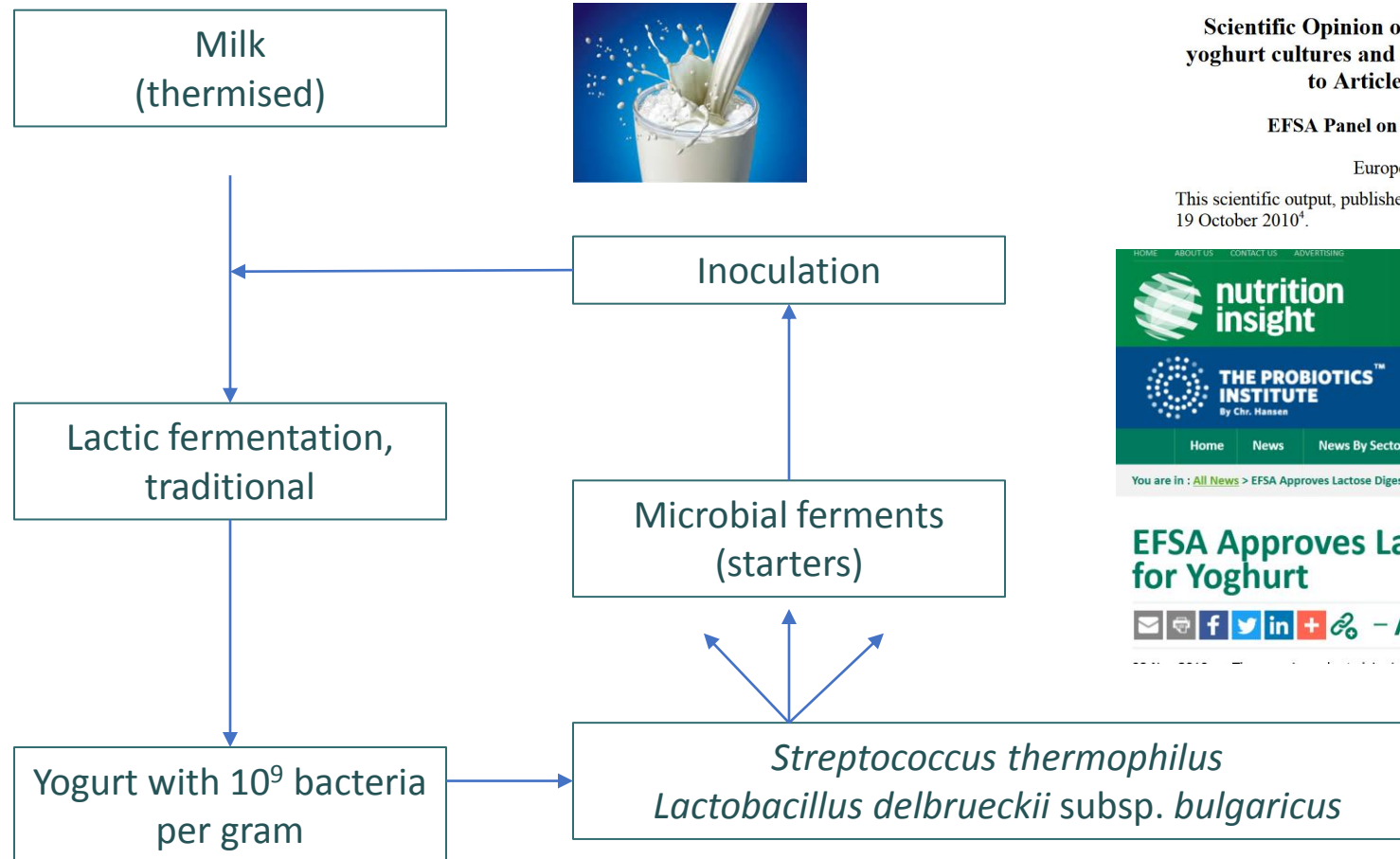
EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

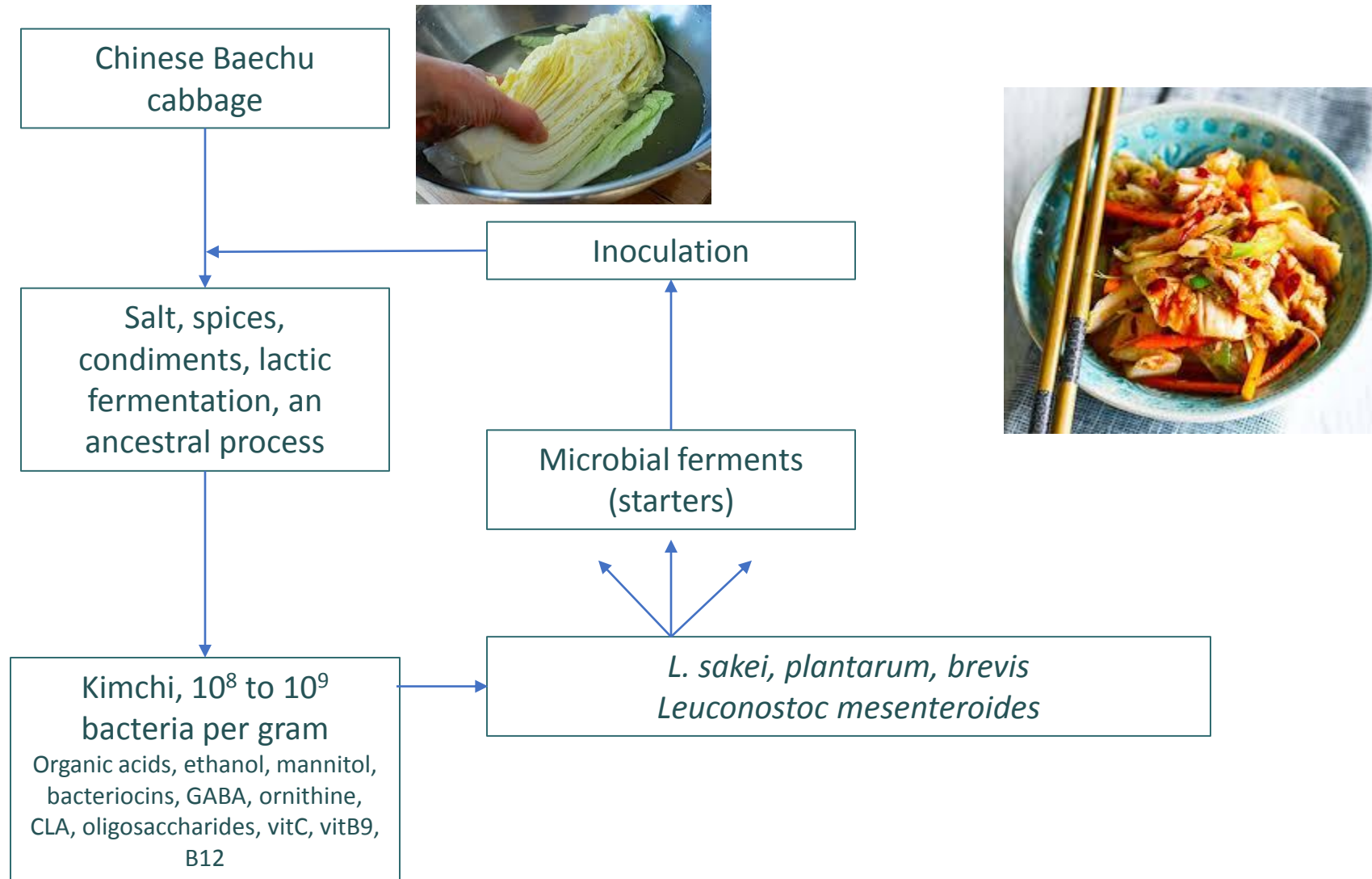
This scientific output, published on 12 January 2011, replaces the earlier version published on 19 October 2010⁴.



EFSA Approves Lactose Digestion Claim for Yoghurt



➤ kimchi, a traditional probiotic vegetable fermented food



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



Online Submissions: <http://www.wjgnet.com/esps/>
wjg@wjgnet.com
doi:10.3748/wjg.v18.i30.4012

World J Gastroenterol 2012 August 14; 18(30): 4012-4018
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
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BRIEF ARTICLE

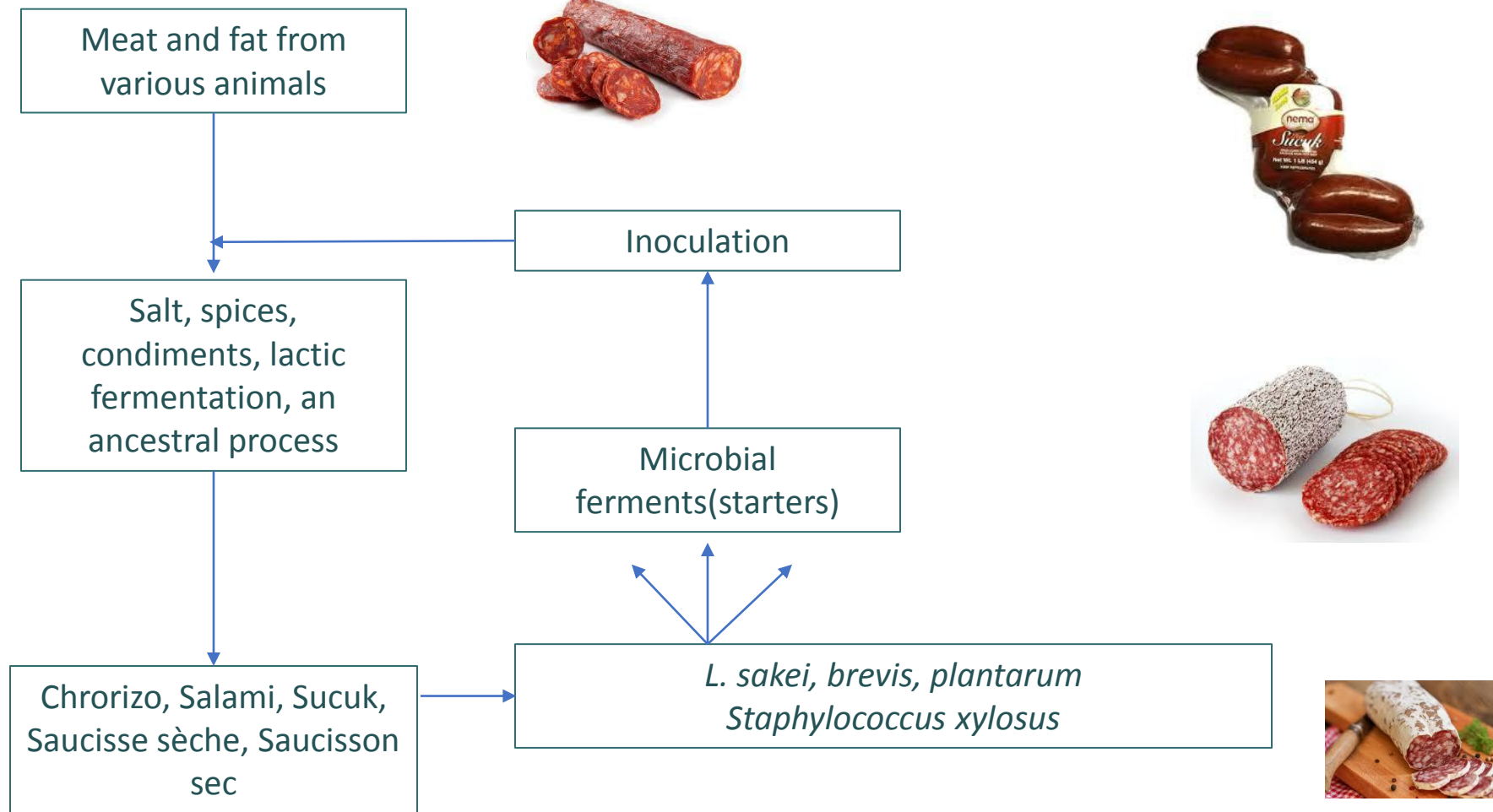
Clinical trial: *Lactobacillus plantarum* 299v (DSM 9843) improves symptoms of irritable bowel syndrome

Philippe Ducrotté, Prabha Sawant, Venkataraman Jayanthi

Consumption of *L. plantarum*

- ↓ severity of visceral pain
- ↓ defecation frequency
- ↓ bloating

➤ Fermented sausages



➤ *Lactobacillus 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.



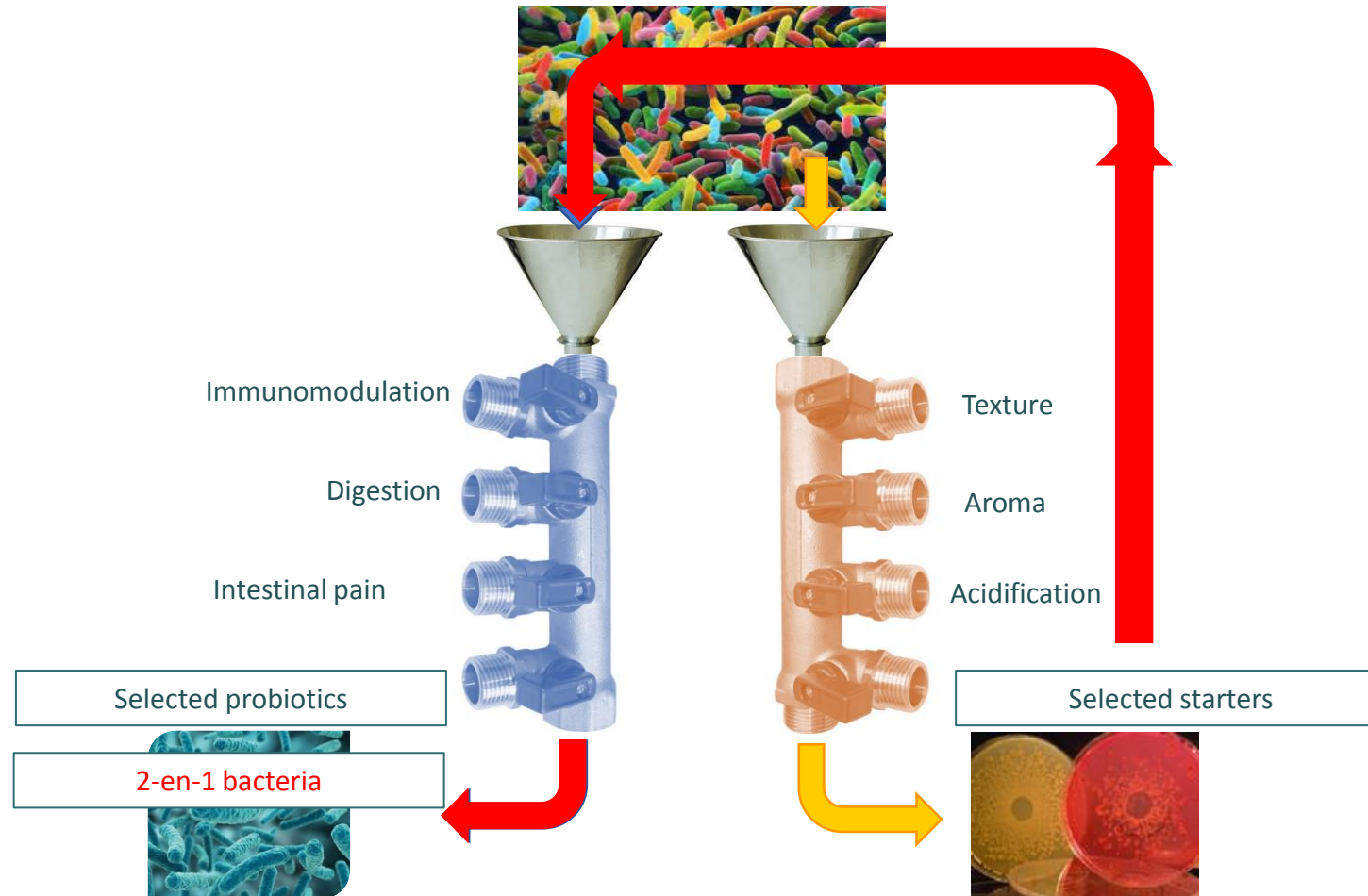
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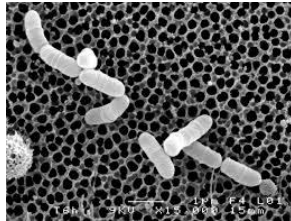
➤ Back to selection of “2-in-1” strains

With both technological and probiotic potential

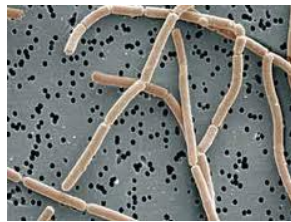


➤ As an example....

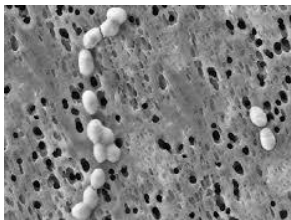
Starters of Emmental cheese



Propionibacterium freudenreichii



Lactobacillus delbrueckii



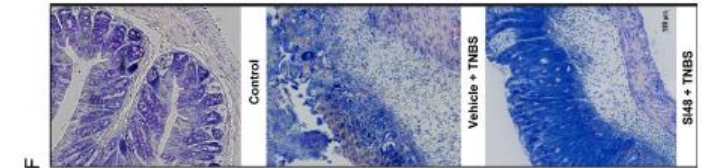
Streptococcus thermophilus

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2010, p. 8259–8264
0099-2240/10/\$12.00 doi:10.1128/AEM.01976-10
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Vol. 76, No. 24

Promising Immunomodulatory Effects of Selected Strains of Dairy Propionibacteria as Evidenced *In Vitro* and *In Vivo*[†]

Benoit Folligné,^{1,2,3,4} Stéphanie-Marie Deutsch,^{5,6} Jérôme Breton,^{1,2,3,4} Fabien J. Cousin,^{5,6,7} Joëlle Dewulf,^{1,2,3,4} Michel Samson,⁸ Bruno Pot,^{1,2,3,4} and Gwénaél Jan^{5,6*}

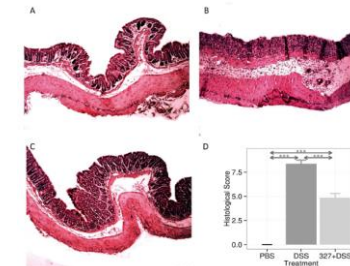


OPEN ACCESS Freely available online



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

Clarissa Santos Rocha^{1,3,4}, Ana Cristina Gomes-Santos², Thais Garcia Moreira², Marcela de Azevedo¹, Tessalia Diniz Luerce¹, Mahendra Mariadassou⁵, Ana Paula Longaray Delamare⁶, Philippe Langella^{3,4}, Emmanuelle Maguin^{3,4}, Vasco Azevedo¹, Ana Maria Caetano de Faria², Anderson Miyoshi^{1*}, Maarten van de Guchte^{3,4,9}



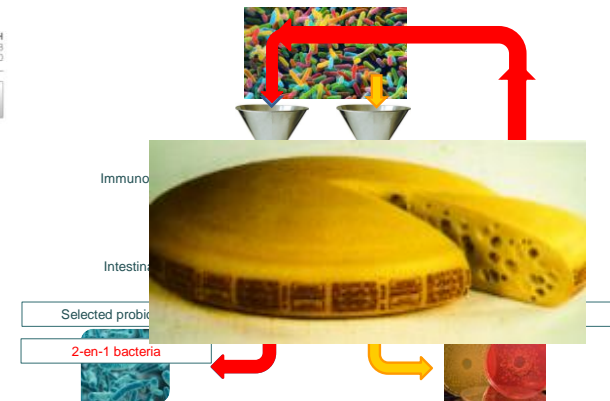
ORIGINAL RESEARCH
published: 24 July 2018
doi: 10.3389/fphys.2018.00060

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Stephen J. Pandolfi,
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University of California, San Diego,
United States
*Correspondence: J. Gagneur

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

Neiké Fernandez^{1†}, Laura Wrzosek^{1†}, Joanna M. Radziwill-Bienkowska², Belinda Ringot-Destrez^{1,4,5}, Marie-Pierre Duviau⁶, Marie-Louise Noordine¹, Valérie Laroute⁶, Véronique Robert¹, Claire Cherbuy¹, Marie-Line Daveran-Mingot⁶, Muriel Coccagn-Bousquet⁶, Renaud Léonard^{1,4,5}, Catherine Robbe-Masselot^{1,4,5}, Françoise Rul¹, Eric Ogier-Denis^{1,4,9}, Muriel Thomas^{1,4} and Muriel Mercier-Bonin^{1†}



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➤ In DSS-induced colitis

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

C57BL6
8th week age



1st Day



5th Day

12th Day

Intragastric gavage

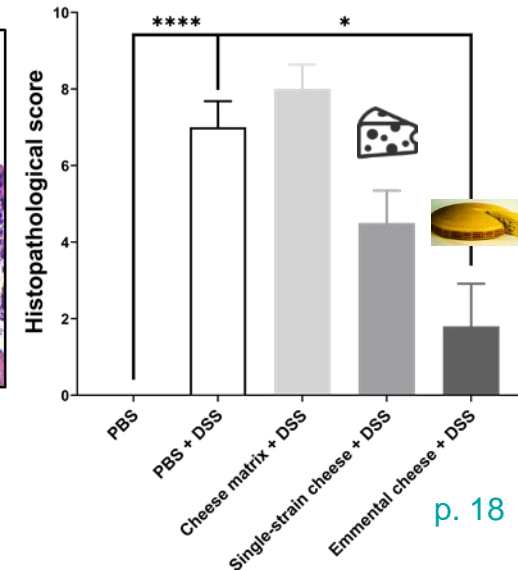
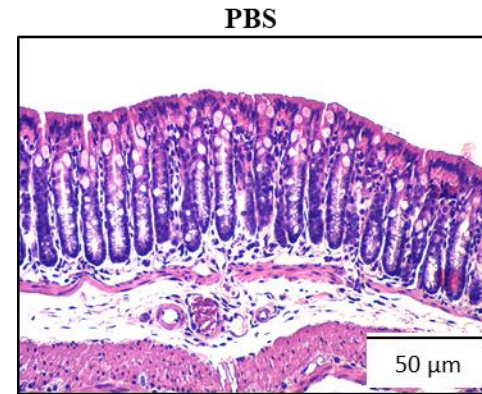
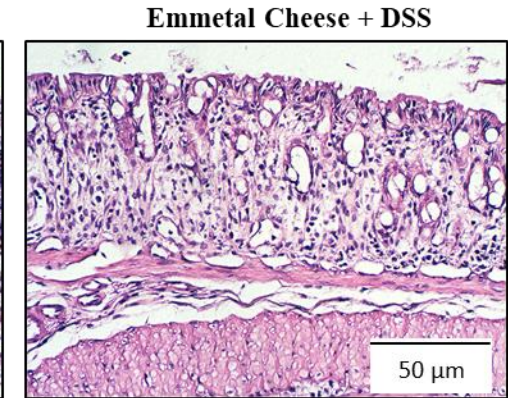
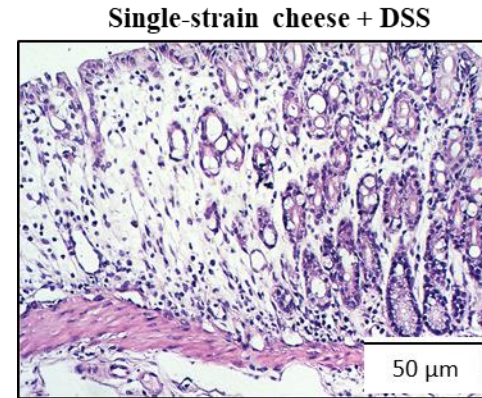
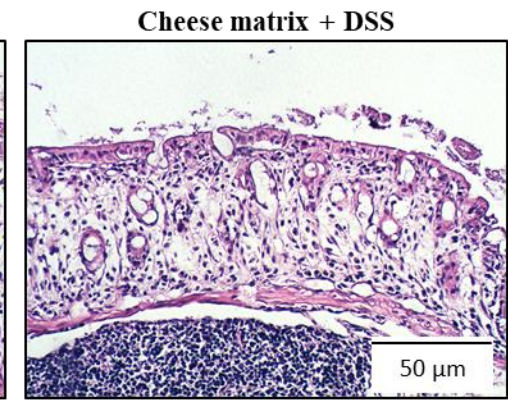
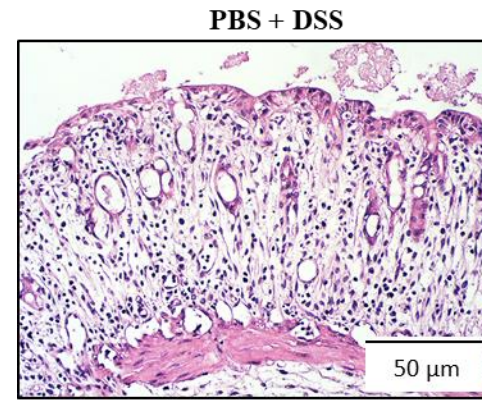
3% (w/v) DSS solution
(36–50 25 kDa)

Gavage 500 μ L
(400 mg cheese in
500 μ L PBS pH 7.4)

Colitis induction

Beginning

Euthanasia



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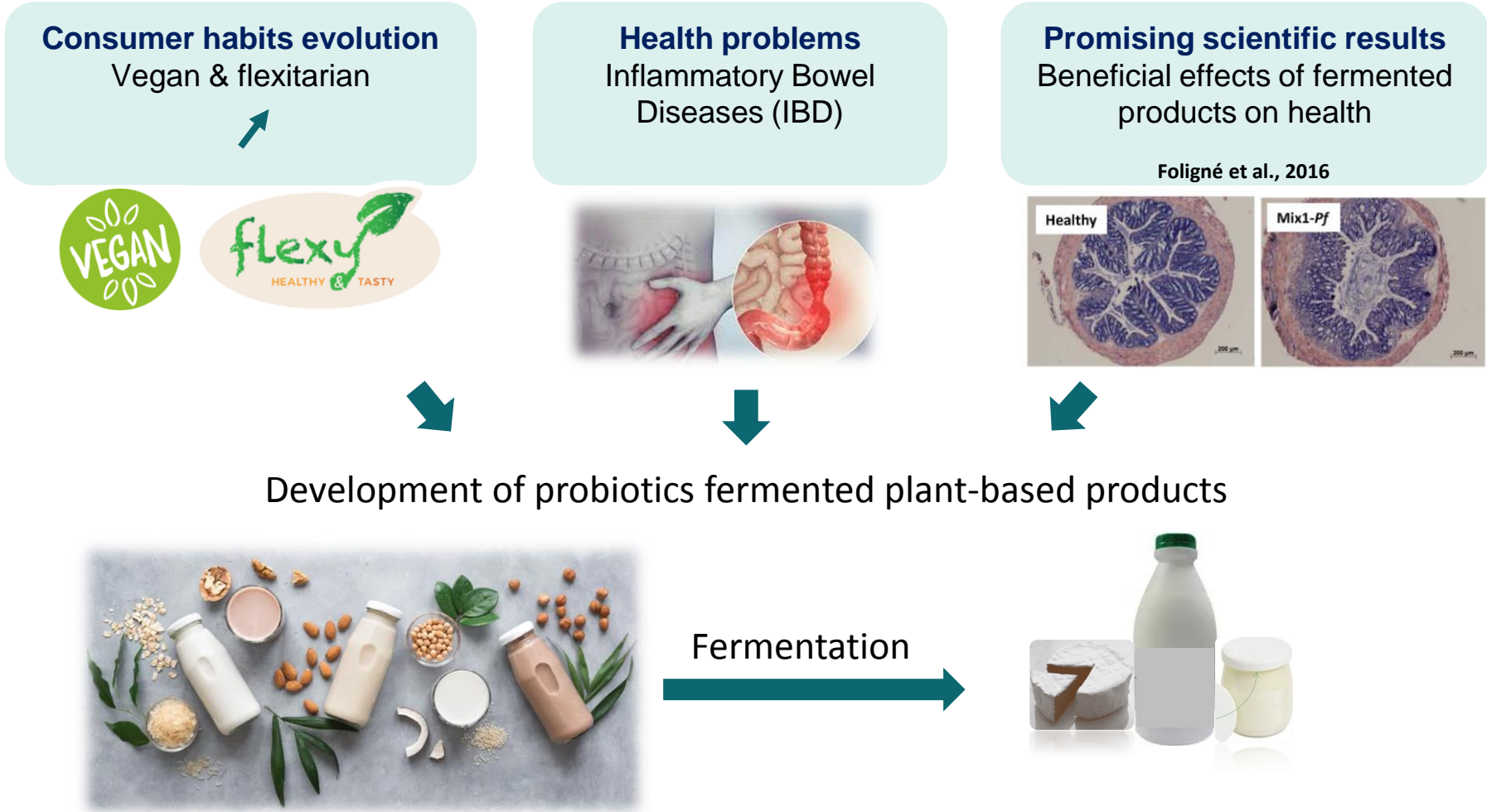
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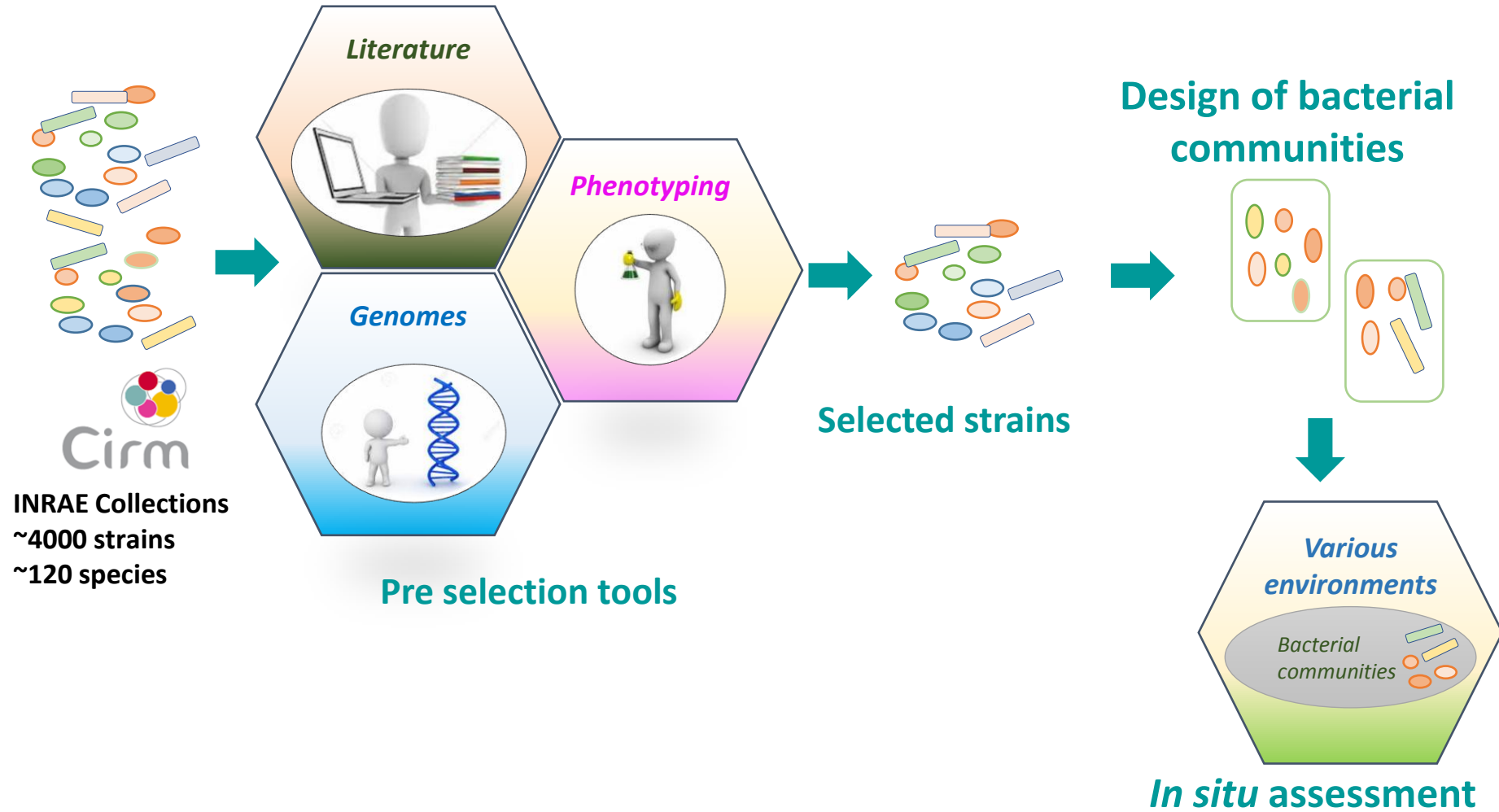
- **But this is a fermented dairy product.....**
- **We now shift to fermented vegetable products**
 - **What do we expect from our « 2-in-1 » bacteria?**
- **Degrade plant sugars: α -galactosidase rather than β -galactosidase?**
- **Degrade plant proteins: enhance bio-availability of N, reduce allergens?**
- **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 Canon¹, Mahendra Mariadassou², Marie-Bernadette Maillard¹, H el ene Falentin¹, Sandrine Parayre¹, Marie-No elle Madec¹, Florence Valence¹, Gw ena le Henry¹, Val erie Laroute³, Marie-Line Daveran-Mingot³, Muriel Cocaign-Bousquet³, Anne Thierry^{1*} and Val erie Gagnaire^{1*}

¹ INRAE, Institut Agro, STLO, Rennes, France, ² INRAE, UR1404 MaIAGE, Jouy-en-Josas, France, ³ Universit e 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:

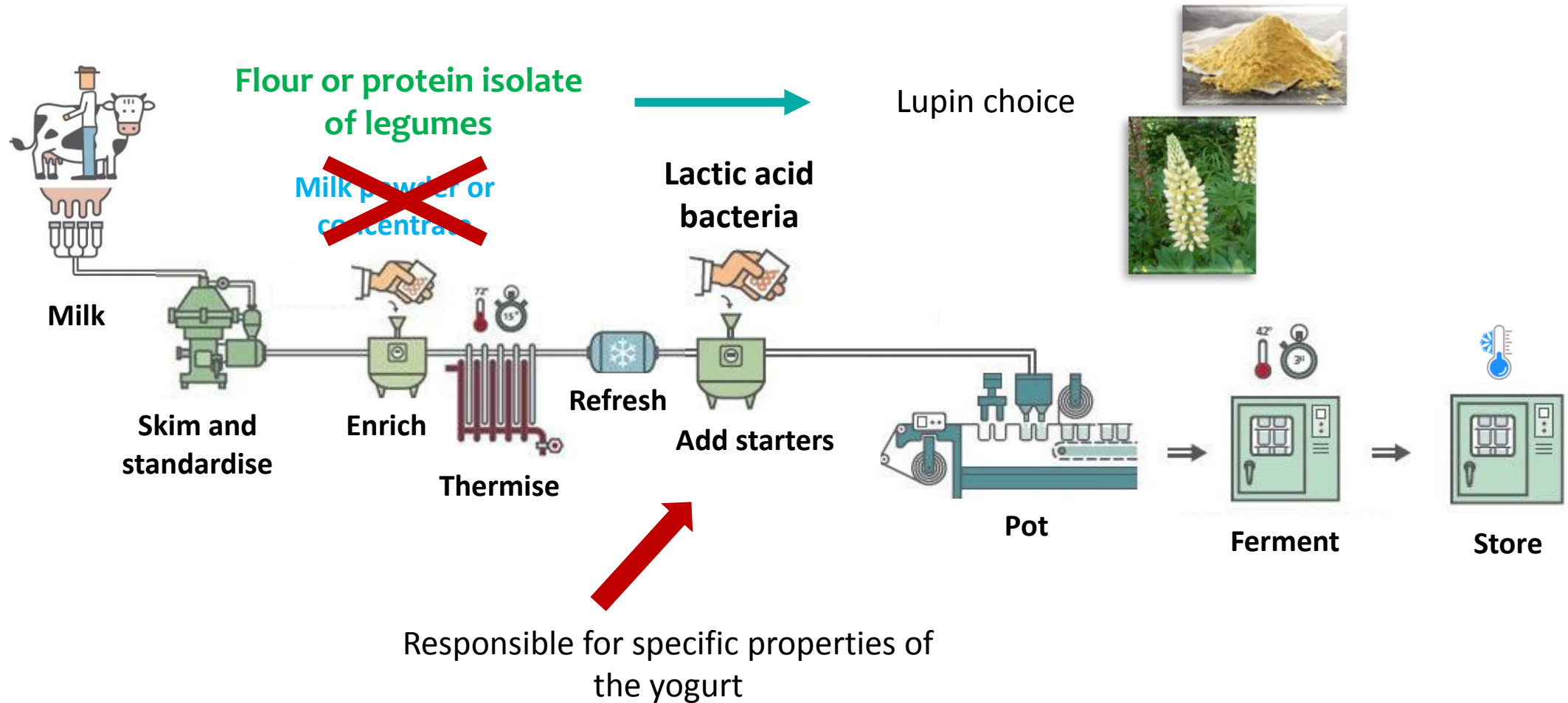
-  stables 
- Legume oligosaccharides  ↘ After fermentation 
- Hexanal  ↘ After fermentation 
- Aroma compound variably produced according to the communities used, sensorial impact 
- « Predigestion » of the proteins variable according to the communities used compared to the unfermented mixes : acceleration 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



➤ Scheme of yogurt and fermented milk production



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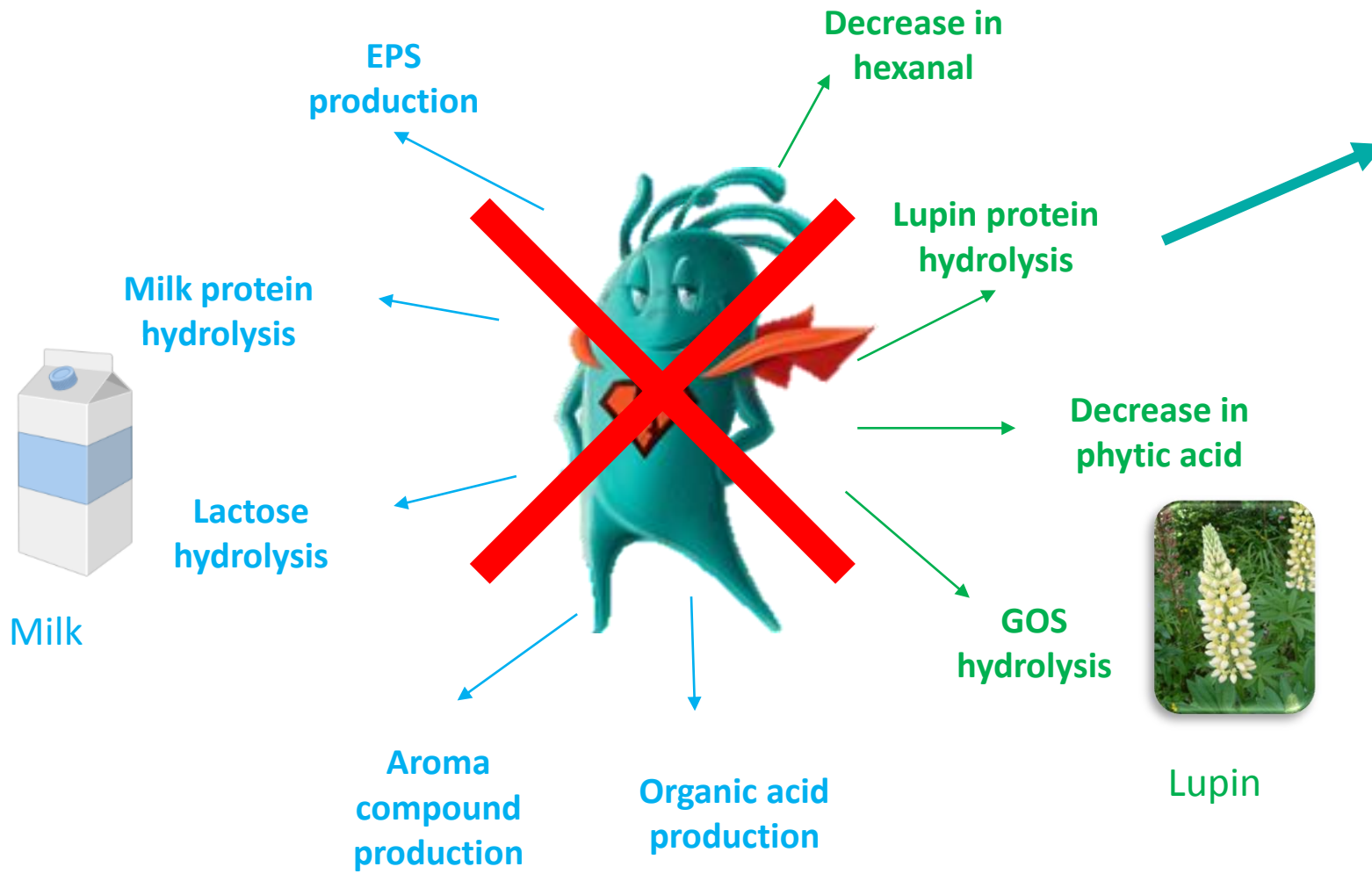


fanny.canon@agrosupdijon.fr



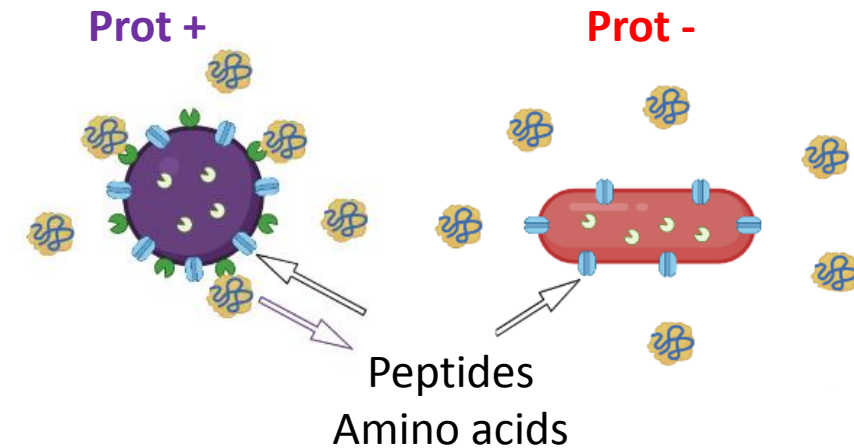
anne.thierry@inrae.fr

➤ 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

5 starter cultures



Monocultures

Cocultures

Levels

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

- F x P
- L x P

Proteolytic activity

+++

++

-

Responses

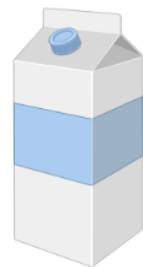
2 fat types



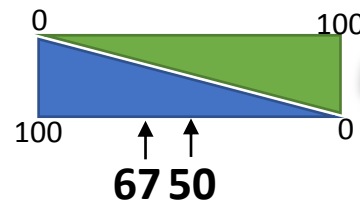
Milk fat
(1,5 %)

Coconut oil
(1,5 %)

2 milk/lupin protein ratios



Milk



Lupin

Bacterial growth

Acidification

Proteolysis

Volatile compounds and organic acid determination

Physical Properties

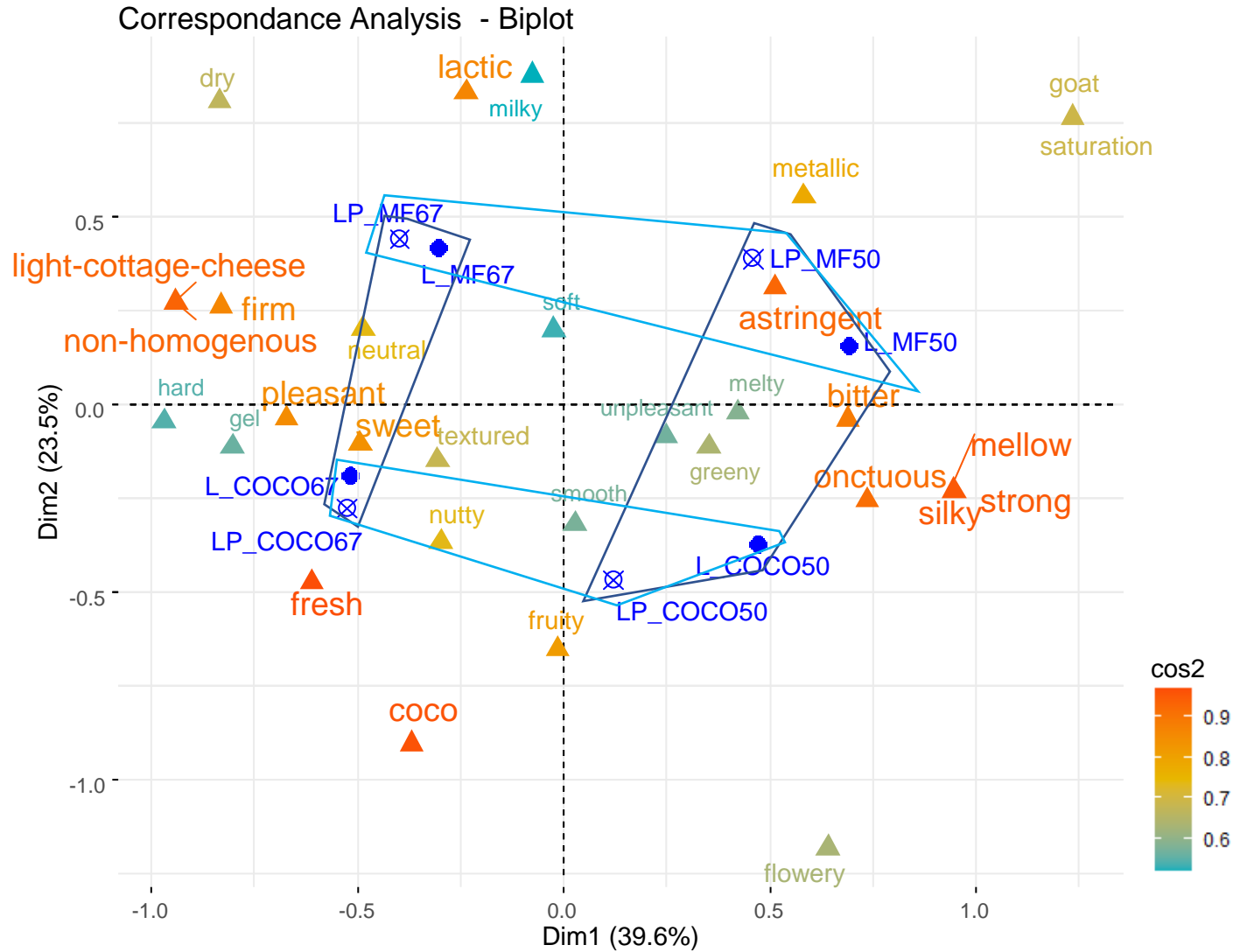
Firmness

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

Untrained panellists



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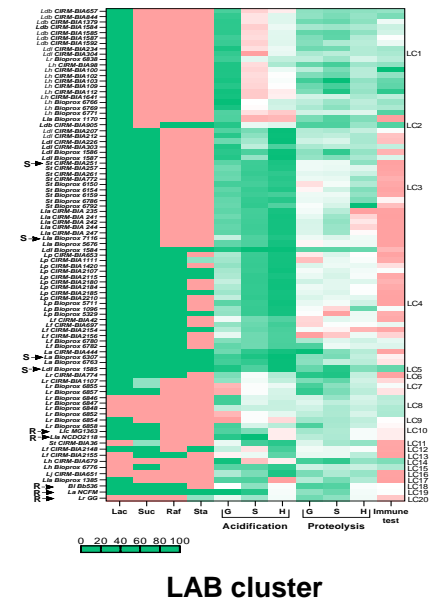
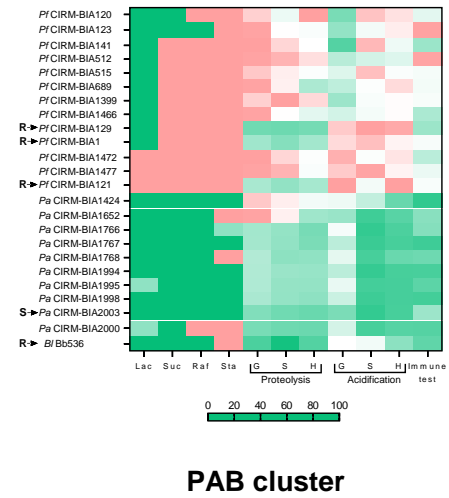
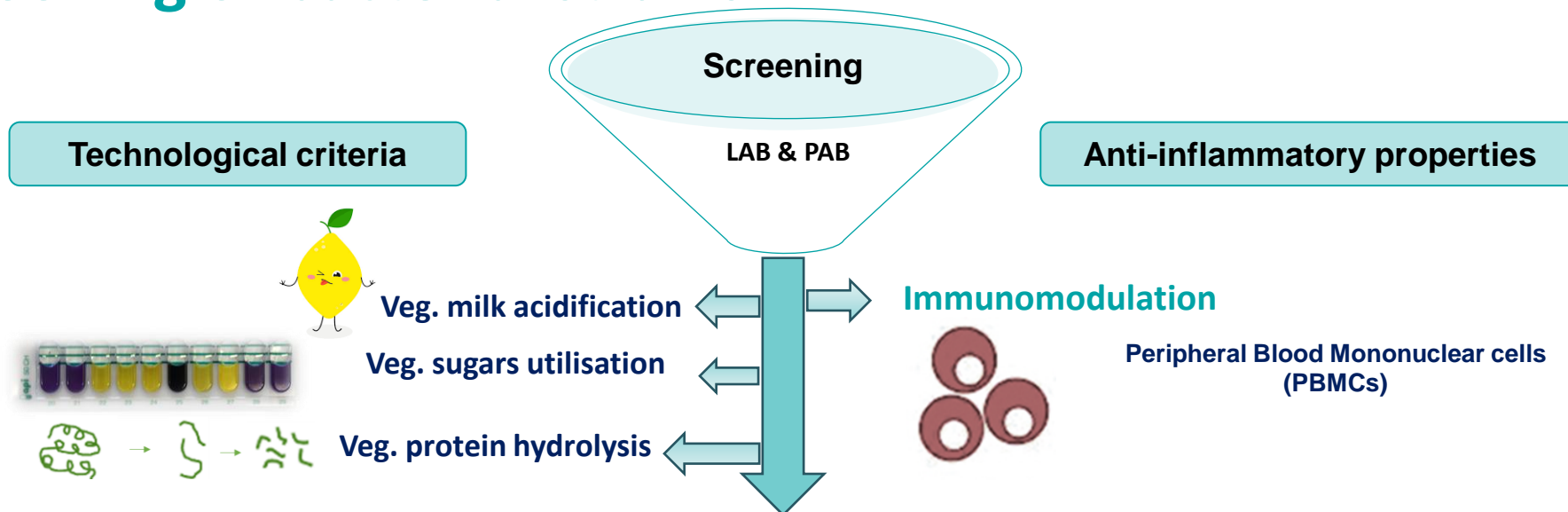
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➤ What about fermented 100% vegetable dairy analogues?



➤ Screening of bacterial strains



➤ Implementation of these strains

WP2 of Localnutleg project

Thank you for your attention

