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## Development of a anti inflammatory cheese

Gwénaél Jan

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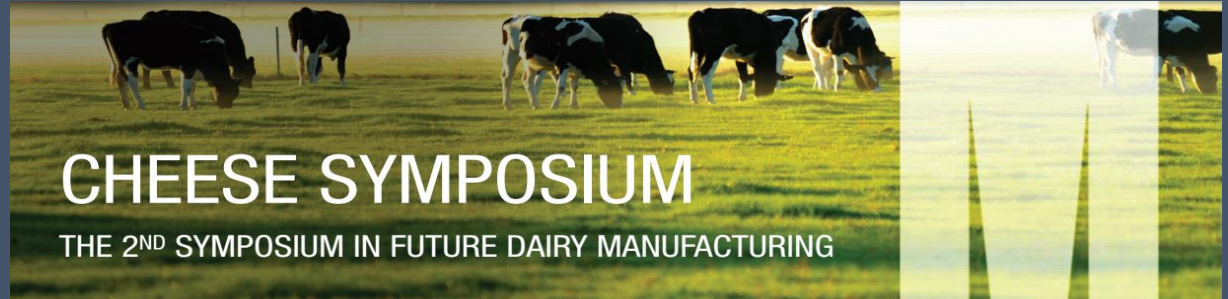
Submitted on 5 Jun 2020

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# Development of an anti-inflammatory cheese



CHEESE SYMPOSIUM

THE 2<sup>ND</sup> SYMPOSIUM IN FUTURE DAIRY MANUFACTURING



**Gwénaél JAN**

UMR 1253 STLO

INRA Agrocampus Ouest, Rennes, France



# Cheese, an old story ...

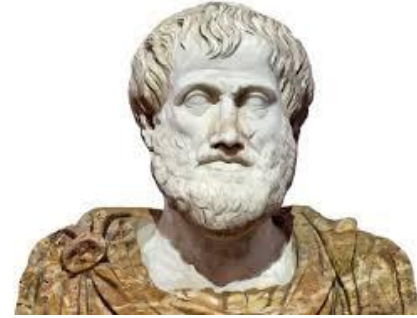


# Sarting at the Neolithic ages



→ Cheese is the most ancient fermented food product

# Aristotle (384 BC –322 BC)



**Kykeon** (Gr. κυκεών, from κυκάω, "to stir, to mix"), an ancient greek recipe made with **wine** and grated **cheese**.

A **magic** medicinal beverage



# Pliny the Elder (born Gaius Plinius Secundus, AD 23–79) « Natural History » «De diversitate caseorum»



Describes a ewe milk cheese from Babales (Lozère, France)



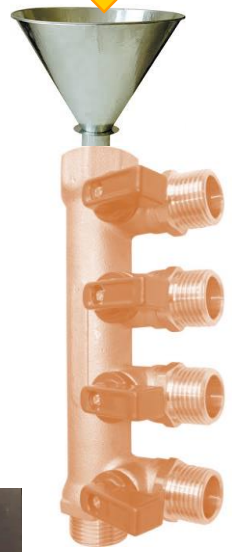
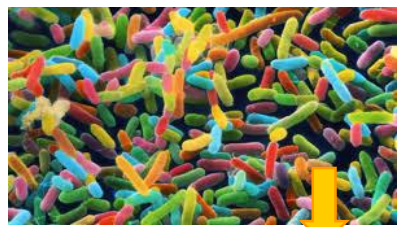
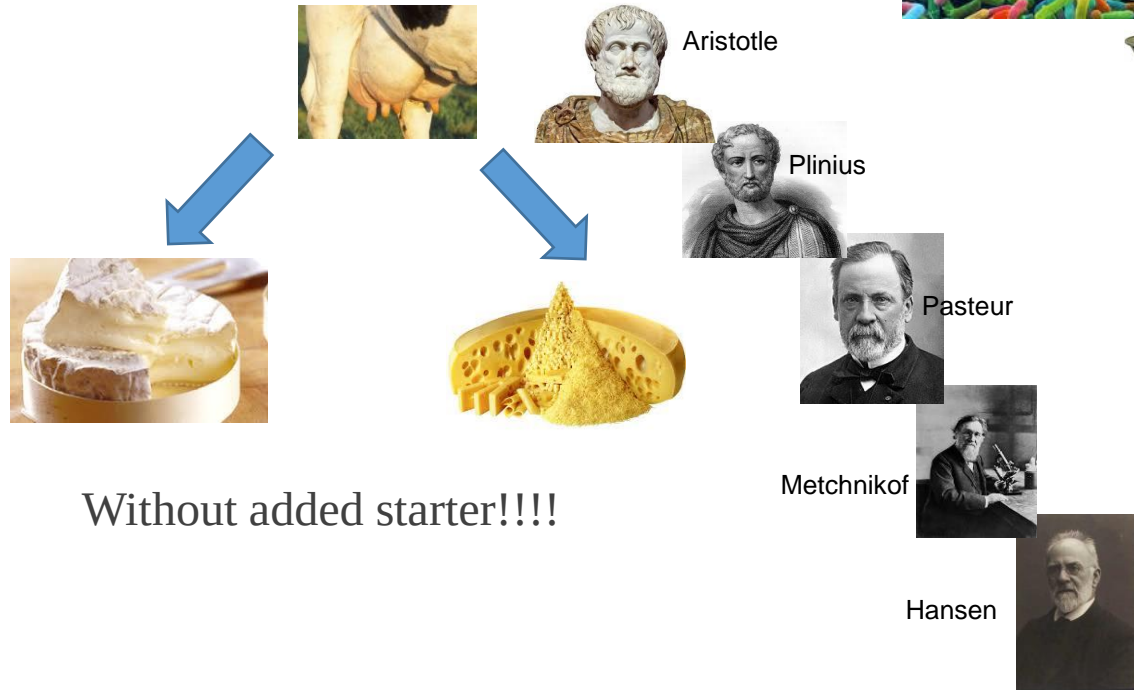
And a cow milk cheese from Arverni (Auvergne, France)



All these cheeses without added starter !!!!

# The technological pathway use to select the bacteria

And now we select the bacteria



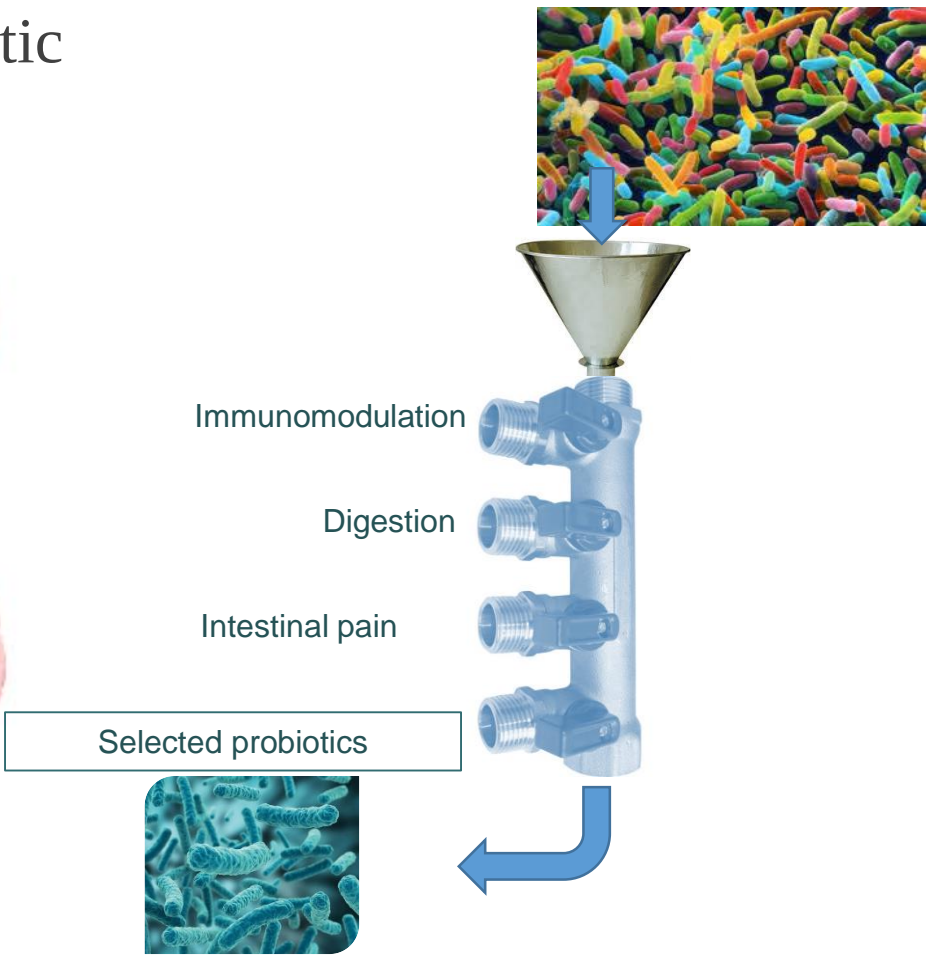
- Texture
- Aroma
- Acidification

Selected starters



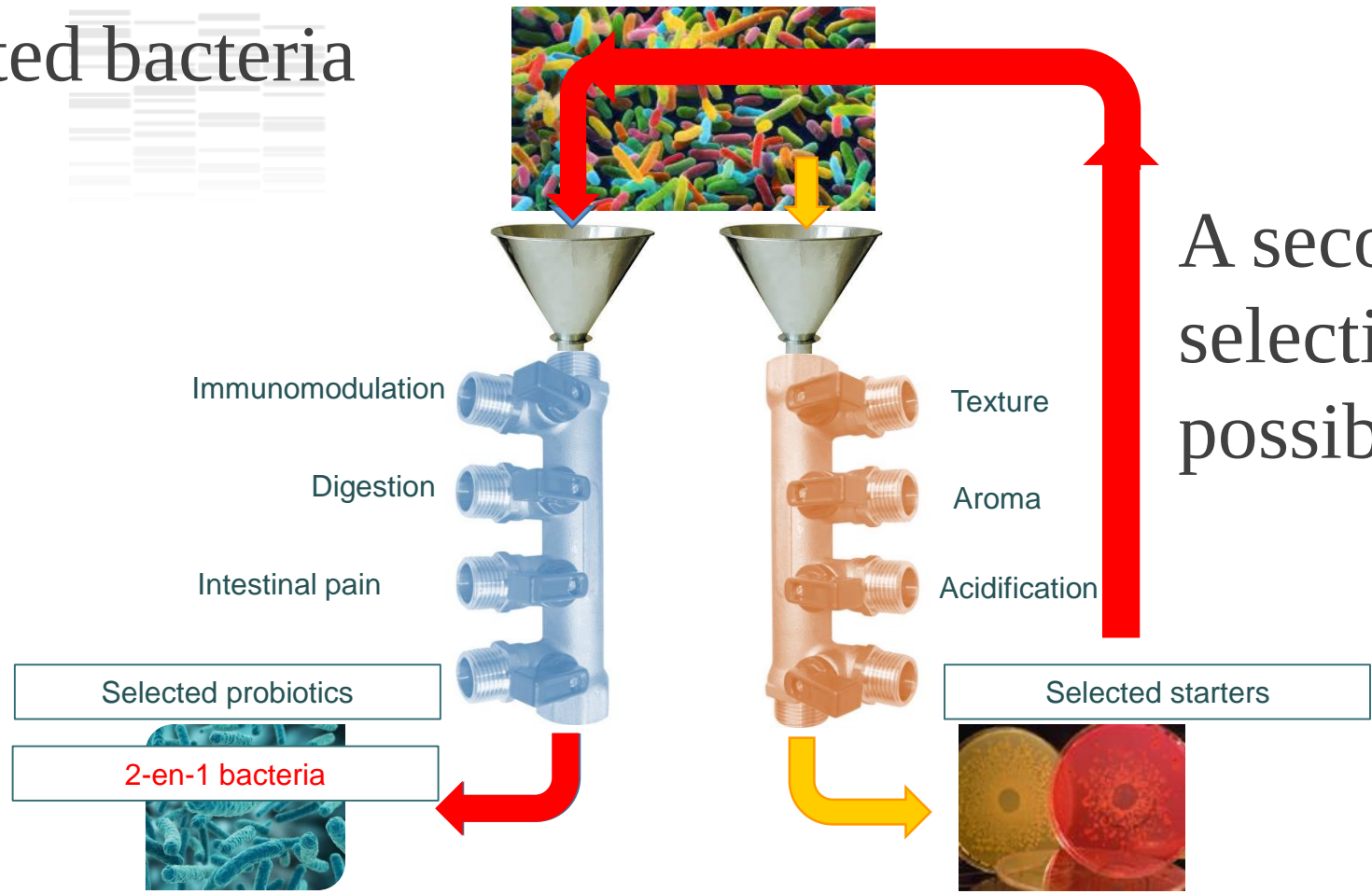
# Other bacteria are selected for probiotic properties

IBS, IBD, atopy, intolerance ...

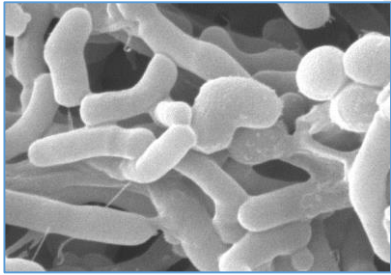




# Selected bacteria



# First example of 2-in-1 bacteria: propionibacteria



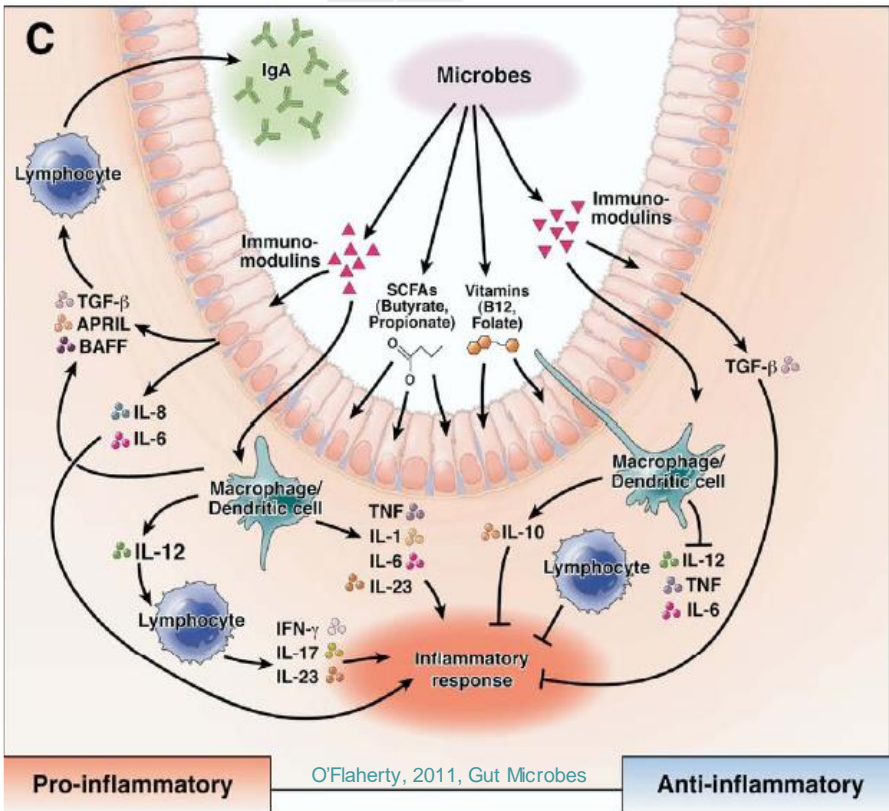
Identity: *Propionibacterium freudenreichii*

Description: Gram+ rods, non-pathogenic, food-grade

Address: everywhere

Profession : cheese maker

# First example of 2-in-1 bacteria: propionibacteria



→ Vitamin producer  
B9, B12



→ Probiotics



→ Food bio-preservative

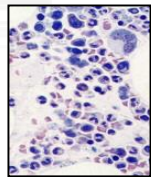
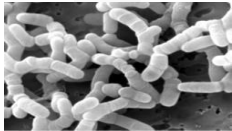


→ Ripening starter



→ SCFAs

# Promising immunomodulators ?



+



IL-10

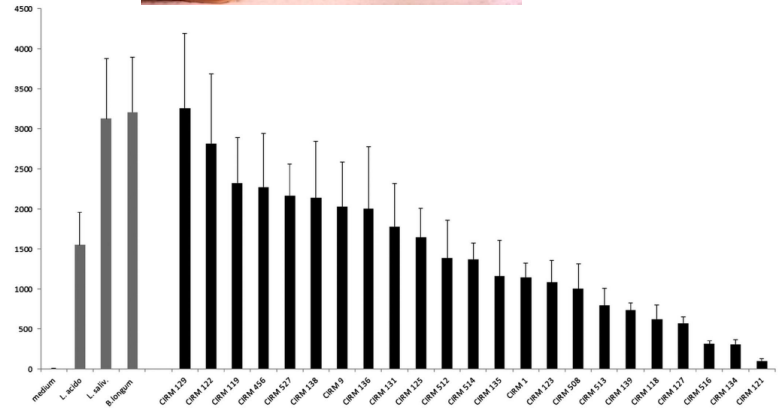


Figure 1 Anti-inflammatory profile of dairy *Propionibacterium freudenreichii* strains assessed on human peripheral blood mononuclear cells (PBMCs). Comparative IL-10 release (in pg/ml) of human PBMCs for 3 reference benchmark probiotic strains (*Lactobacillus acidophilus* NCFM, *L. salivarius* LS33 and *Bifidobacterium longum* BBS36) and for 23 propionibacteria strains (CIRM-BIA, INRA, Rennes, France). Immunocompetent cells were stimulated at multiplicity of infection of 10 for 24 h and supernatants analysed by ELISA. Detailed methodology was previously published.<sup>1</sup>

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2010, p. 8259-8264  
 0999-2240/10/\$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*<sup>†</sup>

Benoît 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 Jai<sup>5,6,\*</sup>

*Institut Pasteur de Lille, Lactic Acid Bacteria & Mucosal Immunity, Center for Infection and Immunity of Lille, 1, Rue du Pr Calmette, BP 245, F-59019 Lille, France<sup>1</sup>; Université Lille Nord de France, F-59000 Lille, France<sup>2</sup>; CNRS, UMR 8204, F-59021 Lille, France<sup>3</sup>; Institut National de la Santé et de la Recherche Médicale, U1019, F-59019 Lille, France<sup>4</sup>; INRA, UMR1253 Science et Technologie du Lait et de l'Oeuf, F-35042 Rennes, France<sup>5</sup>; AGROCAMPUS OUEST, UMR1253 Science et Technologie du Lait et de l'Oeuf, F-35042 Rennes, France<sup>6</sup>; CNIEL Syndicat, 42 Rue de Clérouzet, F-75314 Paris 09, France<sup>7</sup>; and EA 4427 SeRAIC, Faculty of Pharmacy, University of Rennes 1, IFR 140 GEAS, Rennes F-35043, France<sup>8</sup>*

Received 20 August 2010/Accepted 7 October 2010

Immunomodulatory properties of 10 dairy propionibacteria, analyzed on human peripheral blood mononuclear cells (PBMCs), revealed a highly strain-dependent induction of anti-inflammatory cytokine interleukin 10 (IL-10). Two selected strains of *Propionibacterium freudenreichii* showed a protective effect against two models of colitis in mice, suggesting a probiotic potential predicted by immune-based selection criteria for these cheese starter bacteria.



Selected probiotics

➤ Highly strain-dependent



## Tracking the microbiome functionality: focus on Propionibacterium species

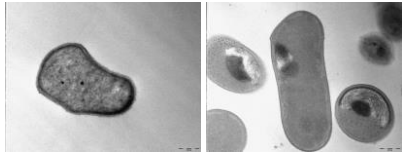
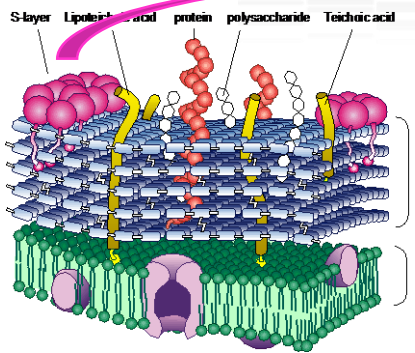
Benoît Foligné, Jérôme Breton, Denis Mater, et al.

Gut 2013 62: 1227-1228 originally published online February 6, 2013  
 doi: 10.1136/gutjnl-2012-304393

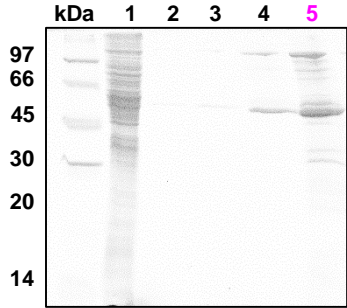


CHEESE SYMPOSIUM  
 THE 2<sup>ND</sup> SYMPOSIUM IN FUTURE DAIRY MANUFACTURING

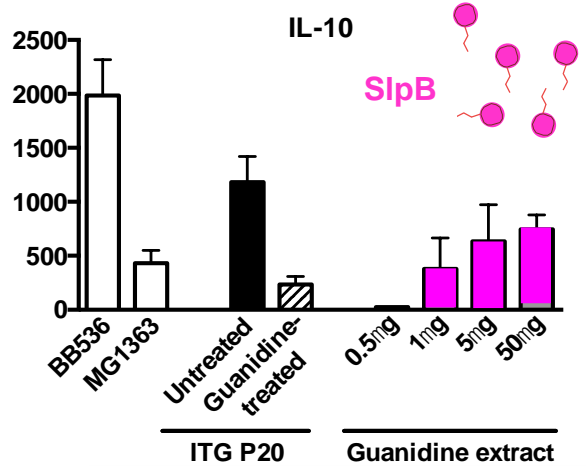
# A key role of a surface protein



Untreated      Guanidine treated

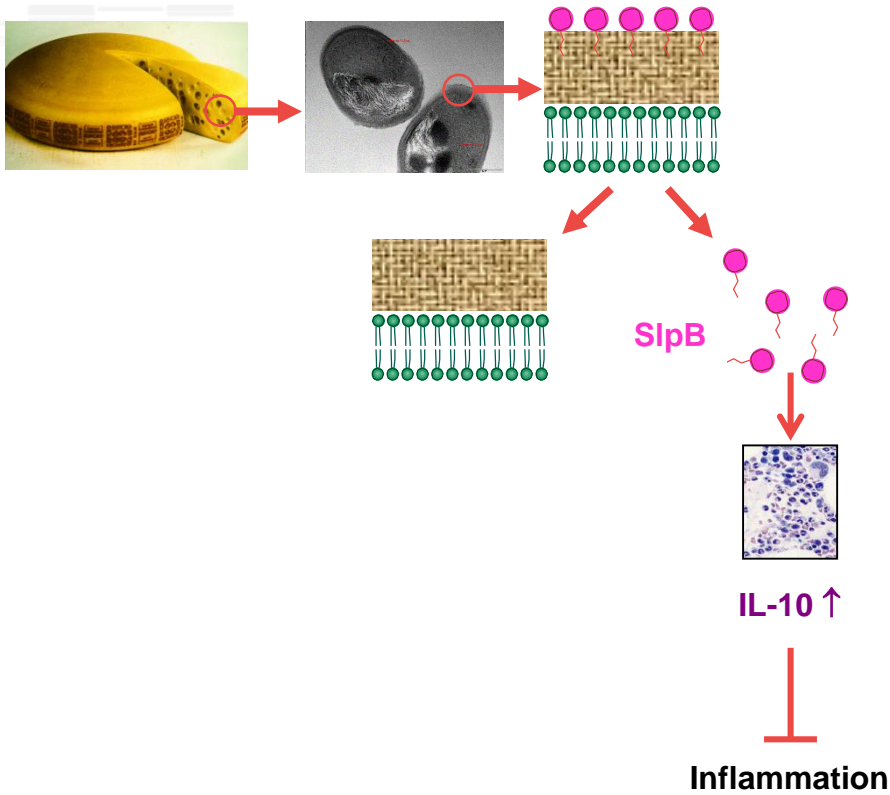


Inactivation of **SlpB** suppresses IL-10 induction

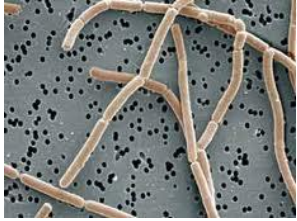


Surface protein **SlpB** induces production of IL-10 in PBMC cells.

# A key role of a surface protein



# Second example of 2-in-1 bacteria



Identity: *Lactobacillus delbrueckii* subsp. *Bulgaricus*

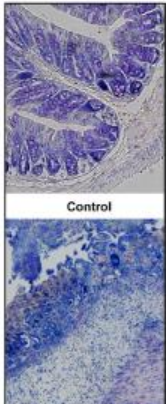
Description: Gram+ bacillus, non-pathogenic, food-grade

Address: mammary gland, raw milk

Playground : yogurt, cheese, with his buddy *S. thermophilus*

Profession : acidification, coagulation, proteolysis, aroma

# Examples of selected bacteria



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*<sup>†</sup>

Benoît Folligné,<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>



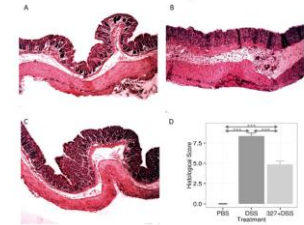
Let's make probiotic cheese!!!

OPEN ACCESS Freely available online



## 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>1,9</sup>,  
 Maarten van de Guchte<sup>3,4,9</sup>



# SURFING

Starter Surface  
 against Inflammation of the Gut



CHEESE SYMPOSIUM  
 THE 2<sup>ND</sup> SYMPOSIUM IN FUTURE DAIRY MANUFACTURING



# A reverse-engineered cheese



Microfiltr. Milk

↓  
Powder 100 g/L

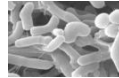


↓  
Cream 150 g/L



↓  
Sterilisation

↓  
Culture *P. freudenreichii*  
30°C, 72 h



↓  
Pre-cheese

UHT Milk



↓  
Culture *L. delbrueckii*  
42°C, 4 h

↓  
Fermented Milk



↓  
Clotting, stirring, cooking,  
moulding, pressing,  
wrapping, ripening



Mol. Nutr. Food Res. 2016, 60, 935–948

DOI 10.1002/mnfr.201500580

935

RESEARCH ARTICLE

Molecular Nutrition  
Food Research

## Combining selected immunomodulatory *Propionibacterium freudenreichii* and *Lactobacillus delbrueckii* strains: Reverse engineering development of an anti-inflammatory cheese

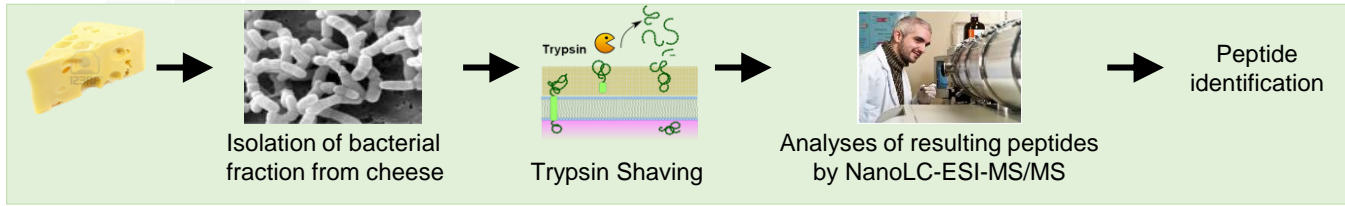
Coline Plé<sup>1</sup>, Jérôme Breton<sup>1</sup>, Romain Richoux<sup>2</sup>, Marine Nurdin<sup>2</sup>, Stéphanie-Marie Deutsch<sup>3,4</sup>,  
Hélène Falentin<sup>3,4</sup>, Christophe Hervé<sup>5</sup>, Victoria Chuat<sup>3,4,6</sup>, Riwanon Lemée<sup>5</sup>,  
Emmanuelle Maguin<sup>7,8</sup>, Gwénaél Jan<sup>3,4</sup>, Maarten Van de Guchte<sup>7,8</sup> and Benoit Foligné<sup>1</sup>



CHEESE SYMPOSIUM

THE 2<sup>ND</sup> SYMPOSIUM IN FUTURE DAIRY MANUFACTURING

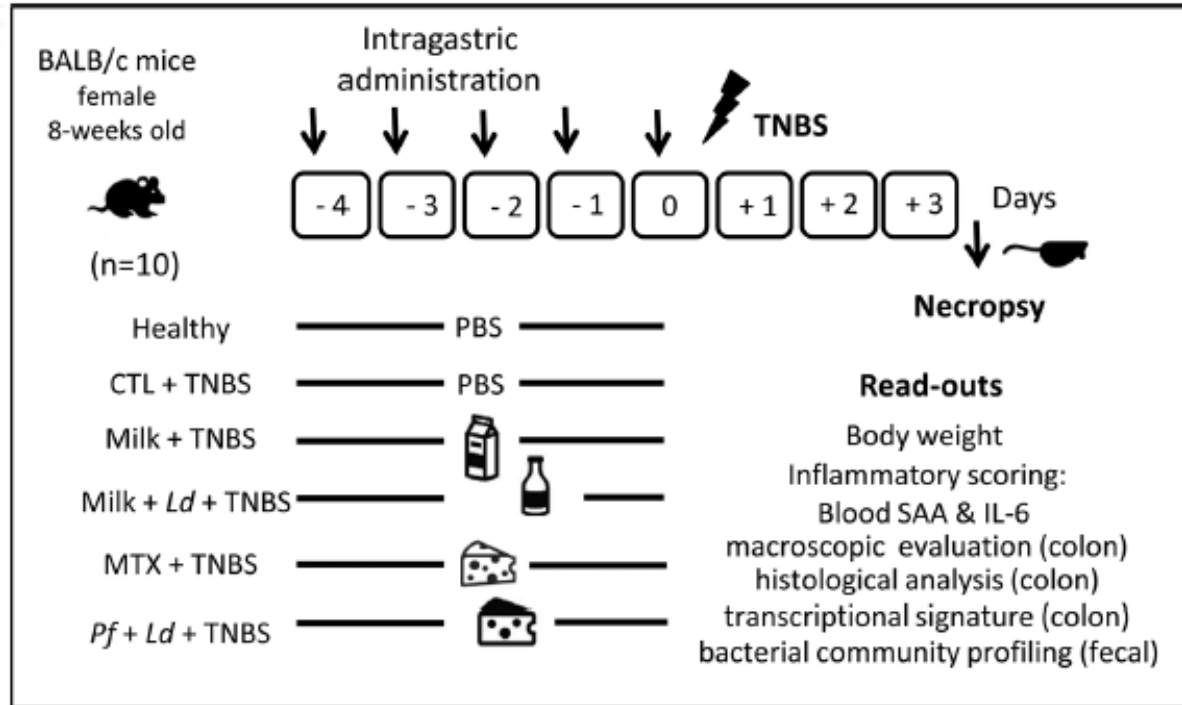
# Key proteins are expressed in the cheese



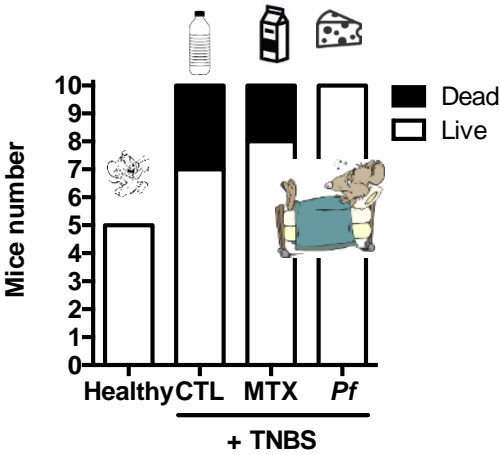
Description	Function	Gene	log(E value)	Peptides	PAI	Group	Sub-group
Surface layer protein B	Cell wall	slpB	-569,10	81	77,3	1	1.1
Surface protein with SLH domain	Cell wall	slpE	-271,21	40	21,5	1	1.2
Surface layer protein A	Cell wall	slpA	-7,48	3	0,6	1	1.3
Internaline A	Miscellaneous	inIA	-838,69	103	26,9	2	2.1
Heat shock protein 20 2	Protein folding	hsp20 2	-69,41	10	19,9	3	3.1
60 kDa chaperonin 2	Protein folding	groL2	-275,04	36	17,0	4	4.1
60 kDa chaperonin 1	Protein folding	groL1	-371,42	49	14,8	4	4.2
Cold shock-like protein CspA	Adaptation to atypical conditions	cspA	-28,17	3	15,5	5	5.1
methyltransferase	Miscellaneous		-66,29	7	15,0	6	6.1
Methylmalonyl-CoA mutase large s	Specific carbohydrate metabolic pathway	mutB	-331,83	43	14,1	7	7.1
Elongation factor Tu	Translation elongation	tuf	-177,14	23	13,6	8	8.1
50S ribosomal protein L7/L12	Ribosomal proteins	rplL	-79,13	10	13,2	9	9.1
Lsr2-like protein	Miscellaneous		-24,65	3	13,0	10	10.1
30S ribosomal protein S9	Ribosomal proteins	rspl	-74,75	12	12,4	11	11.1
30S ribosomal protein S7	Ribosomal proteins	rpsG	-77,54	14	11,5	12	12.1
Hypothetical protein	Protein of unknown function		-183,92	23	10,8	13	13.1
30S ribosomal protein S1	Ribosomal proteins	rpsA	-214,68	27	10,7	14	14.1
ErfK/YbiS/YcfS/YnhG precursor	Cell wall		-120,71	20	10,4	15	15.1
NAD(P)(+) transhydrogenase	Metabolism of coenzymes and prosthetic groups	pntA	-146,56	19	10,4	16	16.1
50S ribosomal protein L35	Ribosomal proteins	rpmI	-17,33	4	10,0	17	17.1



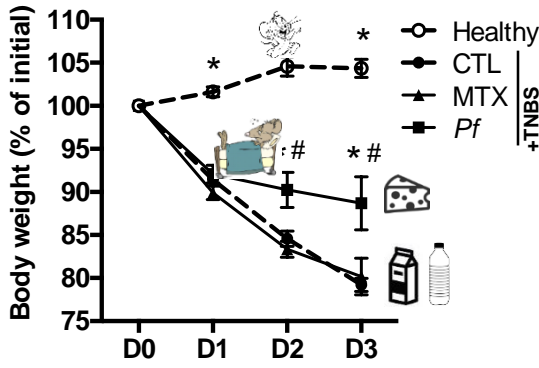
# Experimental design of the animal trial



# Cheese protects at the organism level



Survival

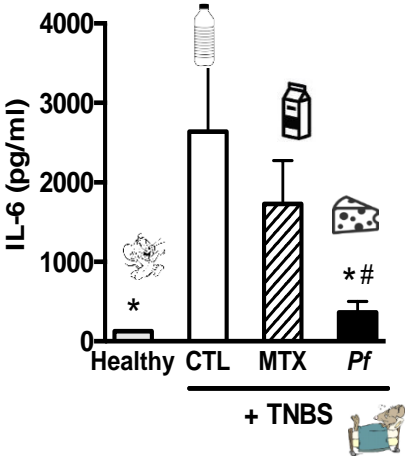


Body weight

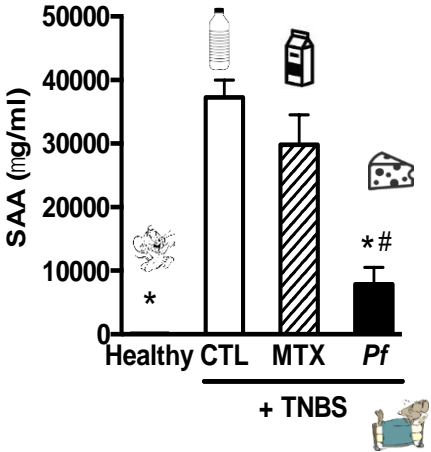
# Cheese protects at the systemic level



## Blood inflammation markers

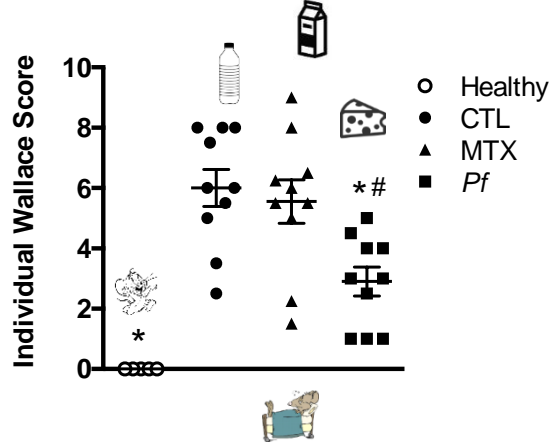
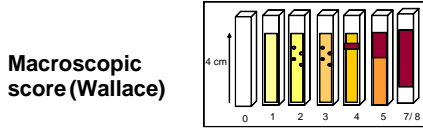
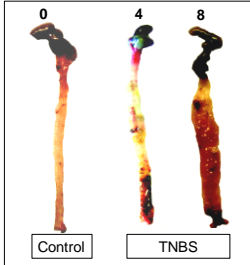
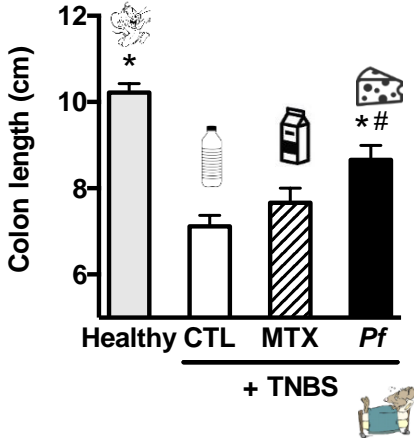


**Interleukin 6**

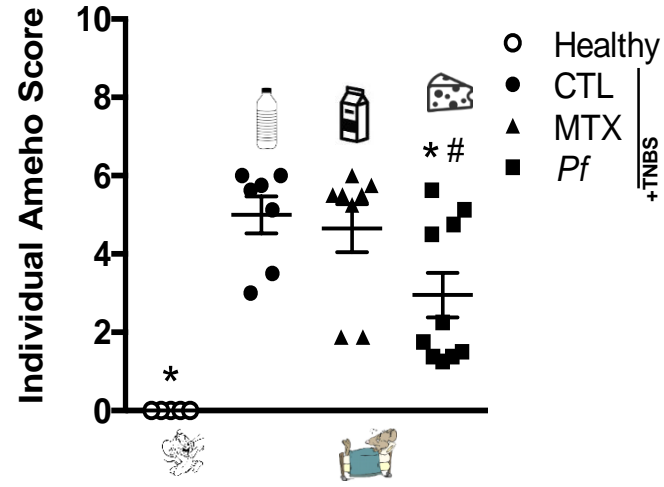
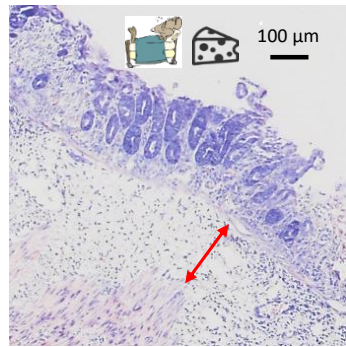
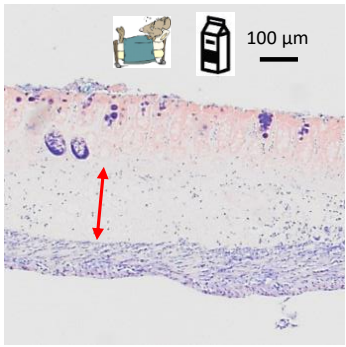
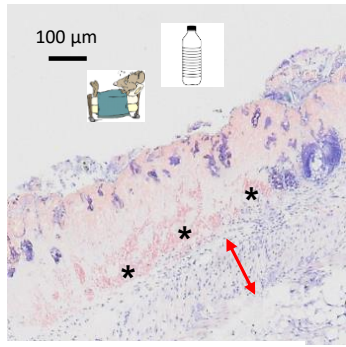
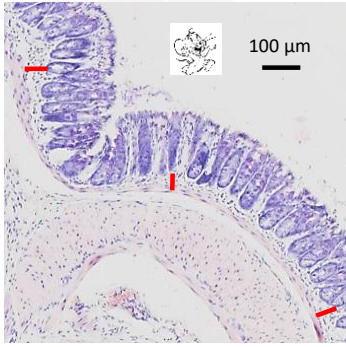


**Serum Amyloid A**

# Cheese protects at organ level

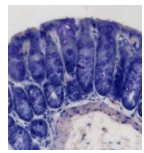
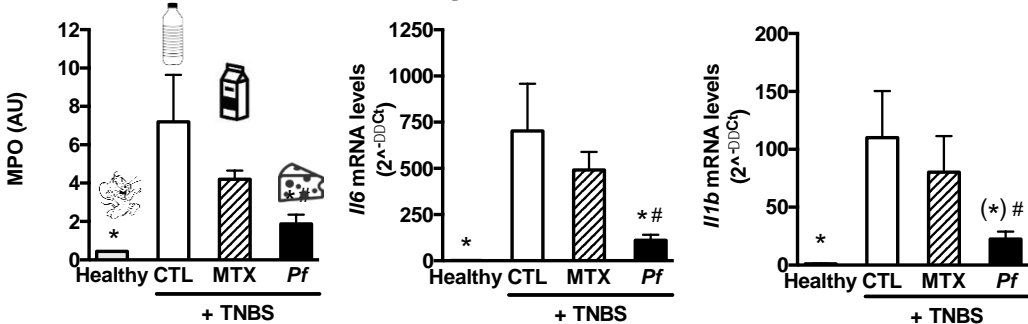


# Cheese protects at the tissue level

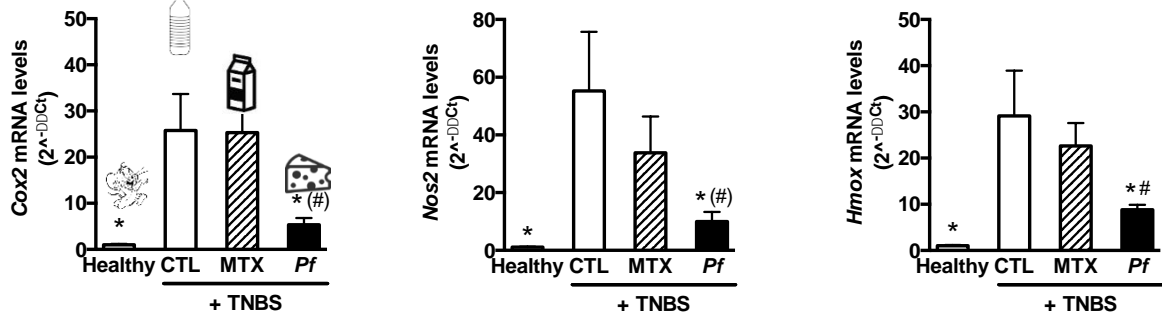


# Cheese protects at the tissue level

## Colonic inflammatory markers



## Colonic oxidative stress markers

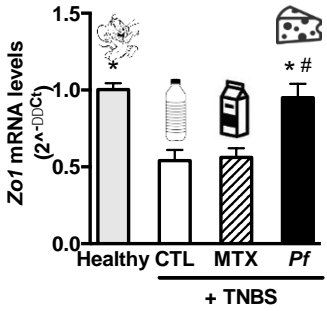


\* p<0.05: vs CTL  
# p<0.05: vs MTX

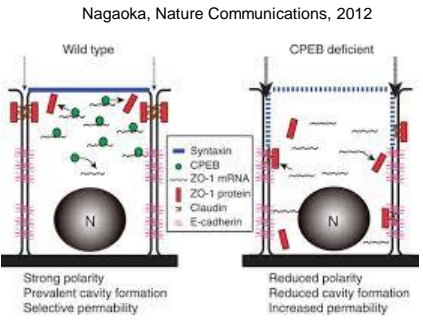
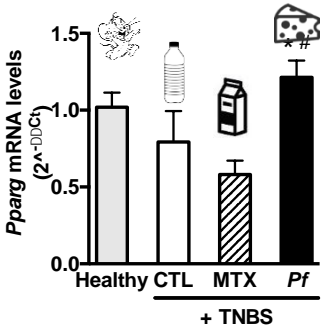


# Cheese protects at the cell level

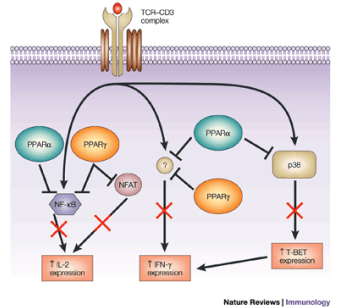
## Colonic epithelial cell markers



\* p<0.05: vs CTL  
# p<0.05: vs MTX



The zona occludens proteins (ZO-1, ZO-2 and ZO-3) : tight junction proteins that function as cross-linkers



Peroxisome proliferator-activated receptors (PPARs) regulate T-cell cytokine production.

# A Chinese story for the end

## Proteomics evidence for kefir dairy in Early Bronze Age China

Yimin Yang<sup>a,b,1</sup>, Anna Shevchenko<sup>c,1</sup>, Andrea Knaust<sup>c</sup>, Idelisi Abuduresule<sup>d</sup>, Wenyang Li<sup>d</sup>,  
Xingjun Hu<sup>d</sup>, Changsui Wang<sup>a,\*</sup>, Andrej Shevchenko<sup>c,\*</sup>

Journal of Archaeological Science 45 (2014) 178–186



**Table 3**

The number of peptides matched to proteins from LAB and yeasts by the proteomics analysis of Xiaohe foods.

Protein name	Gene identifier <sup>a</sup>	Organism <sup>b</sup>	M11	M13a	M22a	M22b	M24	M
<i>LAB proteins</i>								
Glyceraldehyde-3-phosphate dehydrogenase	gi7c336054463	<i>L. kefiranofaciens</i>	7	4	6	5	8	7
Surface layer protein	gi7c336055311	<i>L. kefiranofaciens</i>	3					5
Surface layer protein	gi7c1054802	<i>L. helveticus</i>						2
Phosphopyruvate hydratase	gi7c227893416	<i>Lactobacillus ultunensis</i>	2				2	3
Pyruvate kinase	gi7c336054221	<i>Lactobacillus sp.</i>	2		3	2	2	4
D-lactate dehydrogenase	gi7c104773324	<i>Lactobacillus sp.</i>						2
Fructose-bisphosphate aldolase	gi7c104774377	<i>Lactobacillus sp.</i>						
ATP-dependent protease	gi7c161508175	<i>Lactobacillus sp.</i>						
Conserved hypothetical protein	gi7c260101505	<i>L. helveticus</i>						
Elongation factor Tu	gi7c124377108	<i>L. kefiranofaciens</i>						
Glutamine synthetase	gi7c336053793	<i>L. kefiranofaciens</i>						
<i>Yeasts proteins</i>								
Glyceraldehyde-3-phosphate dehydrogenase 2	gi7c1245703	<i>K. marxianus</i>						
Glyceraldehyde-3-phosphate dehydrogenase 1	gi7c116247787	<i>K. marxianus</i>						
Pyruvate decarboxylase	gi7c416888	<i>K. marxianus</i>						
Enolase 2	gi7c6321968	<i>S. cerevisiae</i>						
Alcohol dehydrogenase 2	gi7c12229579	<i>K. marxianus</i>						
70 kDaI heat shock protein	gi7c172713	<i>Yeast sp.</i>						
Hypothetical protein	gi7c50312181	<i>K. lactis</i>						
Unnamed protein	gi7c2867	<i>K. lactis</i>						
Hypothetical protein	gi7c365984825	<i>Naumovozyma sp.</i>						
Enolase 1	gi7c260944716	<i>Candida lusitaniae</i>						
Hypothetical protein	gi7c156836648	<i>Yeast sp.</i>						
40S ribosomal protein S9	gi7c213402101	<i>Yeast sp.</i>						
tsa1p	gi7c401624401	<i>Yeast sp.</i>						
Histone H2b	gi7c6320430	<i>Yeast sp.</i>						2

**A 4000  
years-old  
fermented  
dairy  
product**



AGRO  
CAMPUS  
OUEST



Fermented foods : 30% of our diet

A major source of bacteria : 10<sup>10</sup> per day!

A huge variety of bacteria



INRA  
SCIENCE & IMPACT



Thank  
you for  
your  
attention

Don't ask  
tough  
questions



Houem Rabah

Fillipe do Carmo

Song Huang

Floriane Gaucher



ID2Santé  
Innovation and Development of the health Sector in BRETAGNE

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Valorial  
PERSONNEL L'ALIMENT PLUS INTELLIGENT