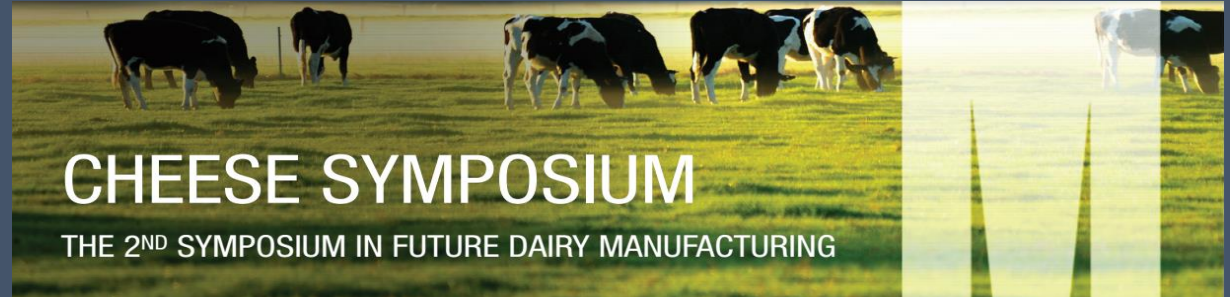




Development of an anti-inflammatory cheese



CHEESE SYMPOSIUM

THE 2ND SYMPOSIUM IN FUTURE DAIRY MANUFACTURING



Gwénaél JAN

UMR 1253 STLO

INRA Agrocampus Ouest, Rennes, France



Cheese, an old story ...

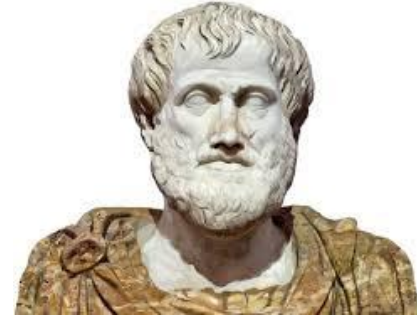


Sarting at the Neolithic ages



→ 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)



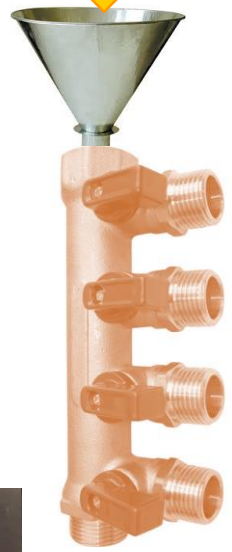
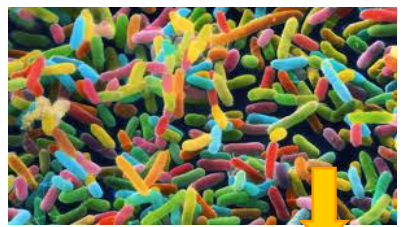
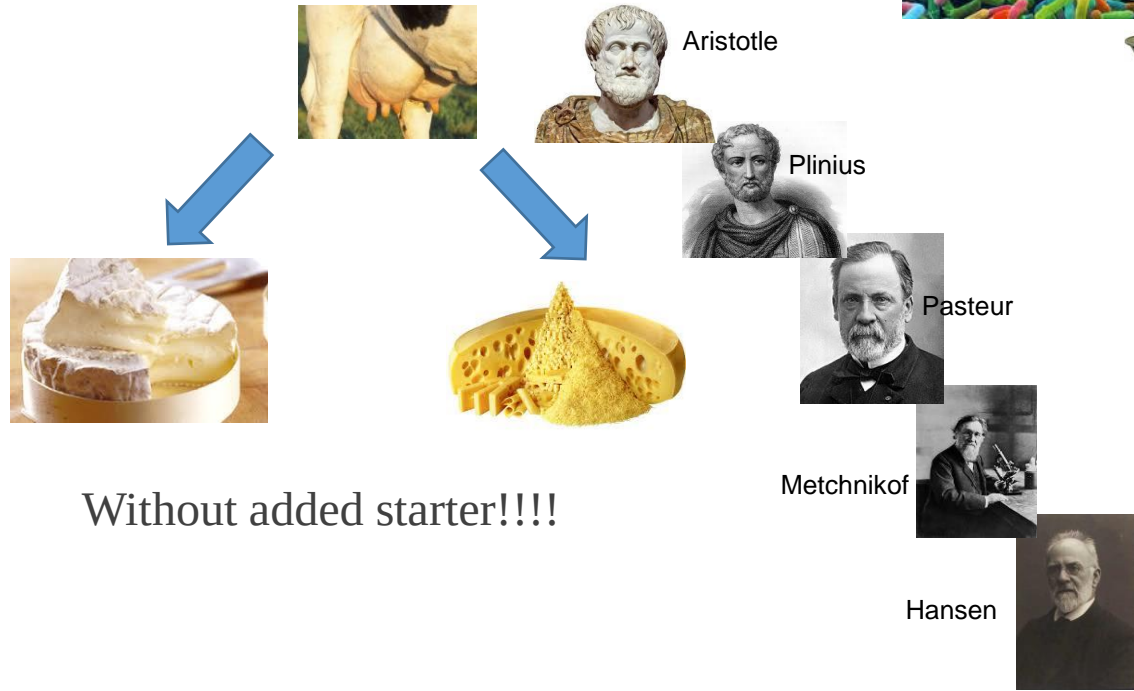
And a cow milk cheese from Arverni (Auvergne, France)



All these cheeses without added starter !!!!

The technological pathway use to select the bacteria

And now we select the bacteria



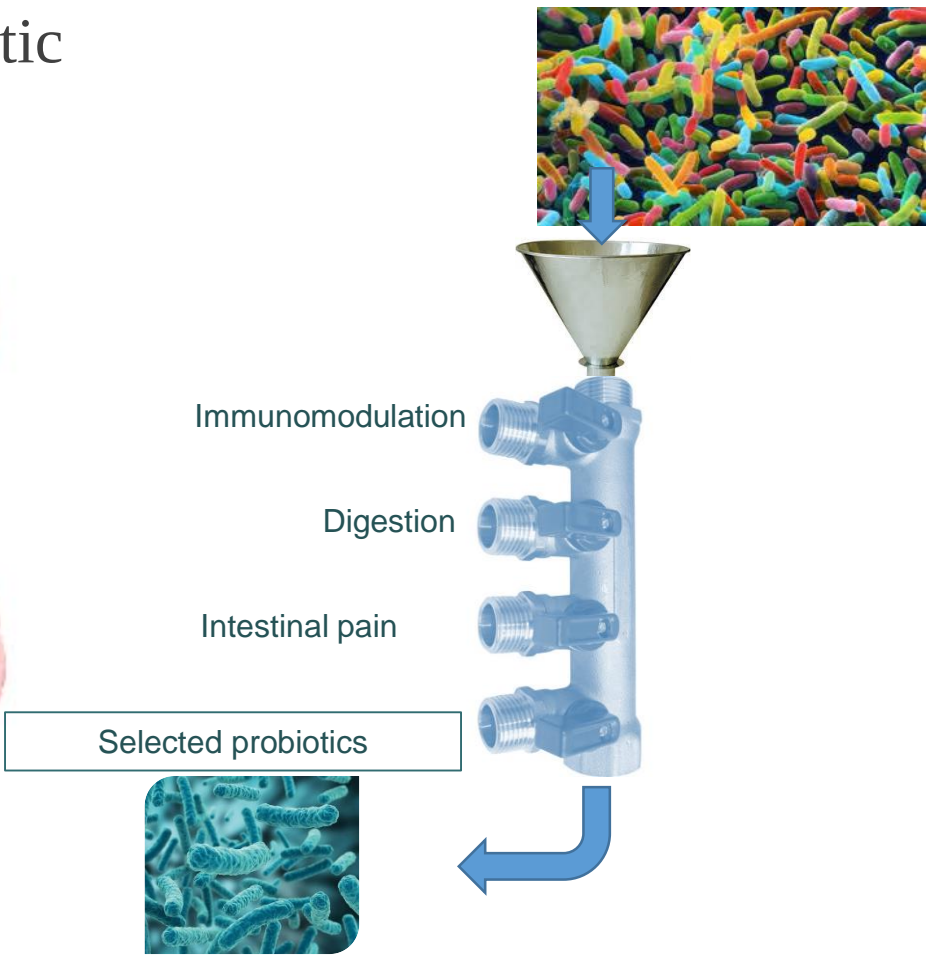
- Texture
- Aroma
- Acidification

Selected starters

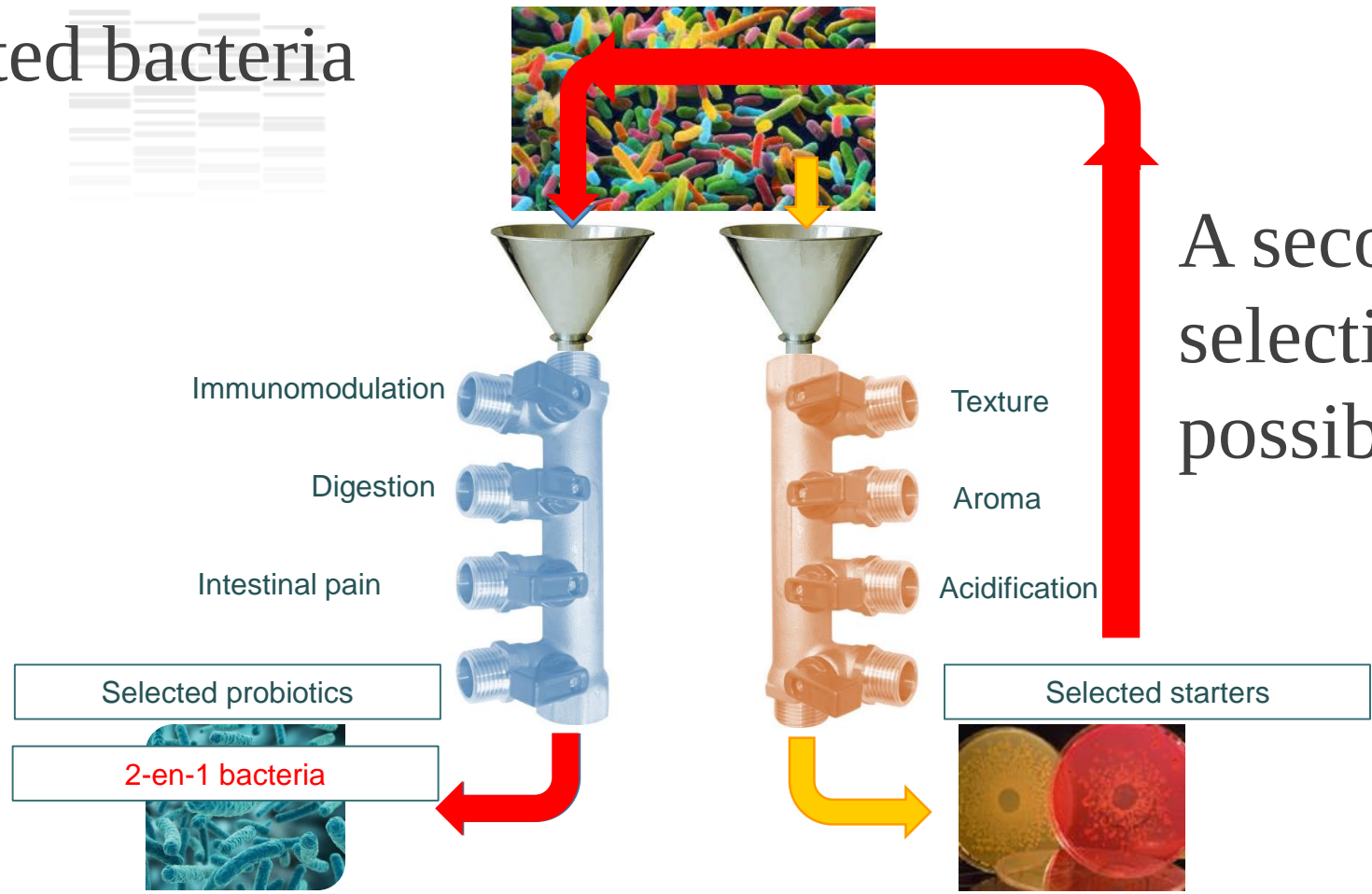


Other bacteria are selected for probiotic properties

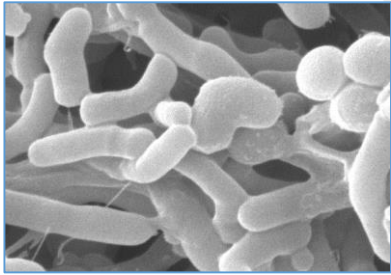
IBS, IBD, atopy, intolerance ...



Selected bacteria



First example of 2-in-1 bacteria: propionibacteria



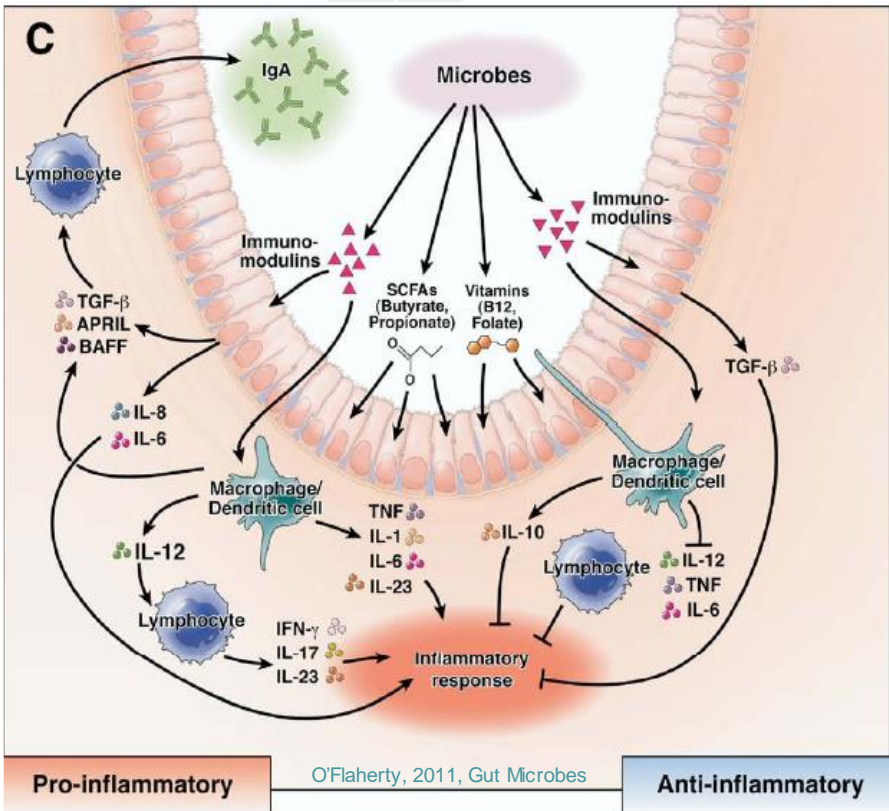
Identity: *Propionibacterium freudenreichii*

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

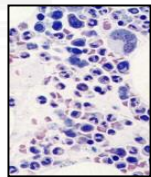
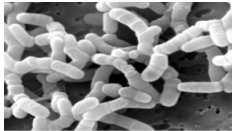


→ Ripening starter



→ SCFAs

Promising immunomodulators ?



+



IL-10

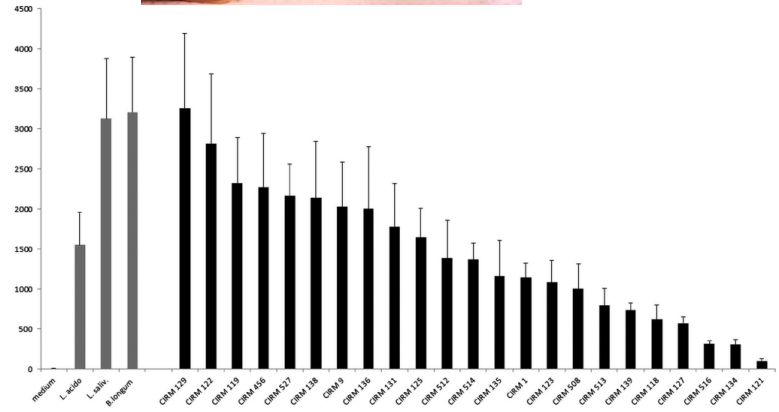


Figure 1 Anti-inflammatory profile of dairy *Propionibacterium freudenreichii* strains assessed on human peripheral blood mononuclear cells (PBMCs). Comparative IL-10 release (in pg/ml) of human PBMCs for 3 reference benchmark probiotic strains (*Lactobacillus acidophilus* NCFM, *L. salivarius* LS33 and *Bifidobacterium longum* BB536) 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.¹

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*[†]

Benoît Foligné,^{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 Jai^{5,6,*}

Insitut 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, France¹; Université Lille Nord de France, F-59000 Lille, France²; CNRS, UMR 8204, F-59021 Lille, France³; Institut National de la Santé et de la Recherche Médicale, U1019, F-59019 Lille, France⁴; INRA, UMR1253 Science et Technologie du Lait et de l'Oeuf, F-35042 Rennes, France⁵; AGROCAMPUS OUEST, UMR1253 Science et Technologie du Lait et de l'Oeuf, F-35042 Rennes, France⁶; CNIEL Syndicat, 42 Rue de Clérouzet, F-75314 Paris 09, France⁷; and EA 4427 SeRAIC, Faculty of Pharmacy, University of Rennes 1, IFR 140 GEAS, Rennes F-35043, France⁸

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

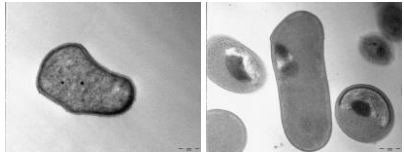
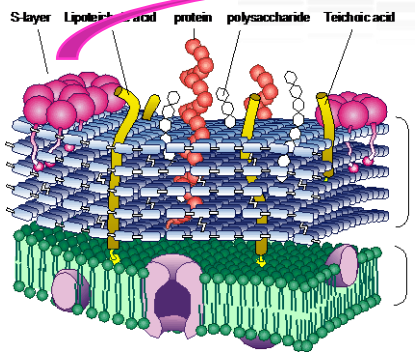
Benoît Foligné, Jérôme Breton, Denis Mater, et al.

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

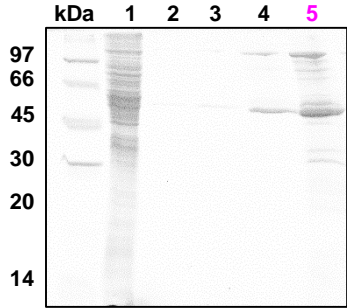


CHEESE SYMPOSIUM
 THE 2ND SYMPOSIUM IN FUTURE DAIRY MANUFACTURING

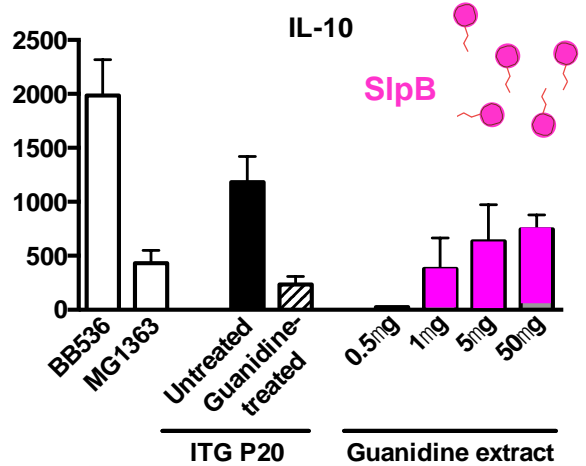
A key role of a surface protein



Untreated Guanidine treated

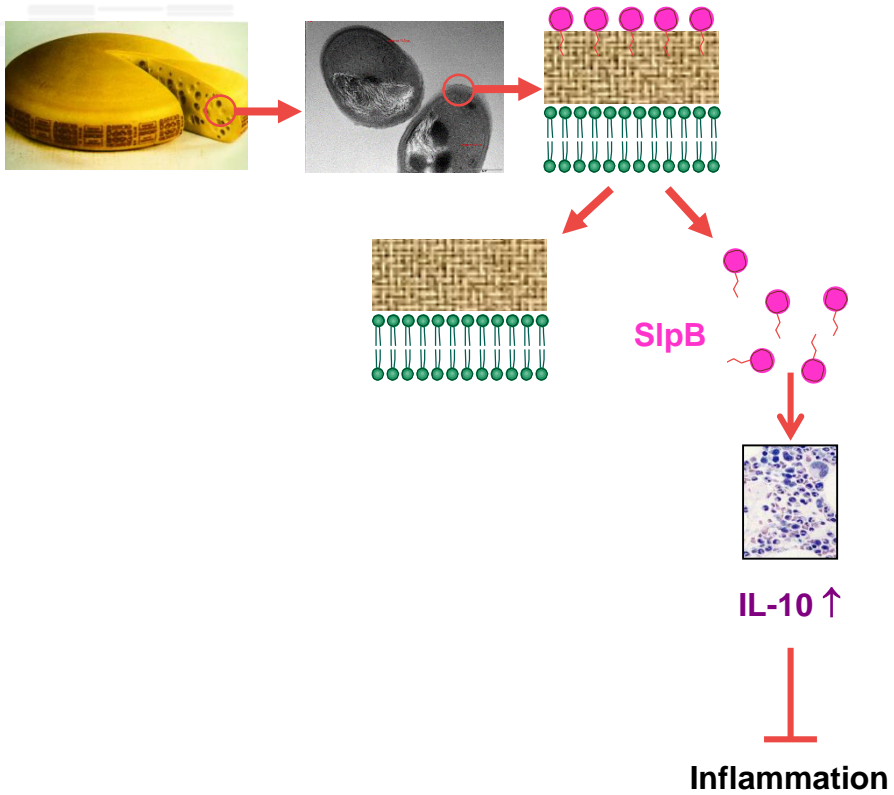


Inactivation of **SlpB** suppresses IL-10 induction

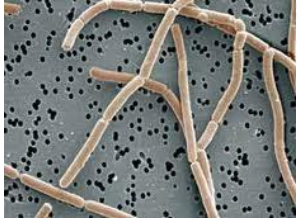


Surface protein **SlpB** induces production of IL-10 in PBMC cells.

A key role of a surface protein



Second example of 2-in-1 bacteria



Identity: *Lactobacillus delbrueckii* subsp. *Bulgaricus*

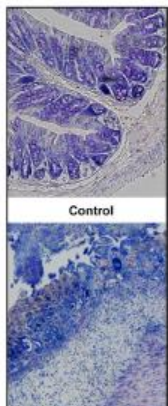
Description: Gram+ bacillus, non-pathogenic, food-grade

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

Benoît 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



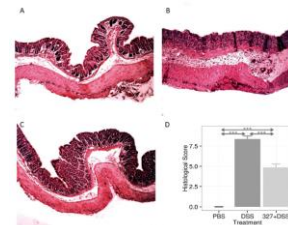
Let's make probiotic cheese!!!

OPEN ACCESS Freely available online



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

Clarissa Santos Rocha^{1,3,4}, Ana Cristina Gomes-Santos², Thais Garcias 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^{1,9}, Maarten van de Guchte^{3,4,9}



SURFING
 Starter Surface
 against Inflammation of the Gut



A reverse-engineered cheese



Microfiltr. Milk

↓
Powder 100 g/L

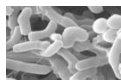


↓
Cream 150 g/L



↓
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

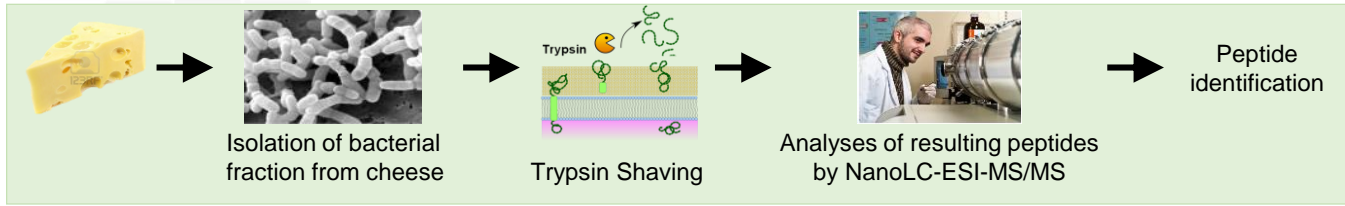
Coline Plé¹, Jérôme Breton¹, Romain Richoux², Marine Nurdin², Stéphanie-Marie Deutsch^{3,4},
Hélène Falentin^{3,4}, Christophe Hervé⁵, Victoria Chuat^{3,4,6}, Riwanon Lemée⁵,
Emmanuelle Maguin^{7,8}, Gwénaél Jan^{3,4}, Maarten Van de Guchte^{7,8} and Benoit Foligné¹



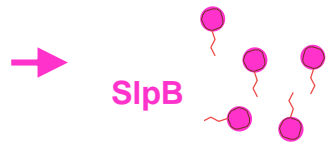
CHEESE SYMPOSIUM

THE 2ND SYMPOSIUM IN FUTURE DAIRY MANUFACTURING

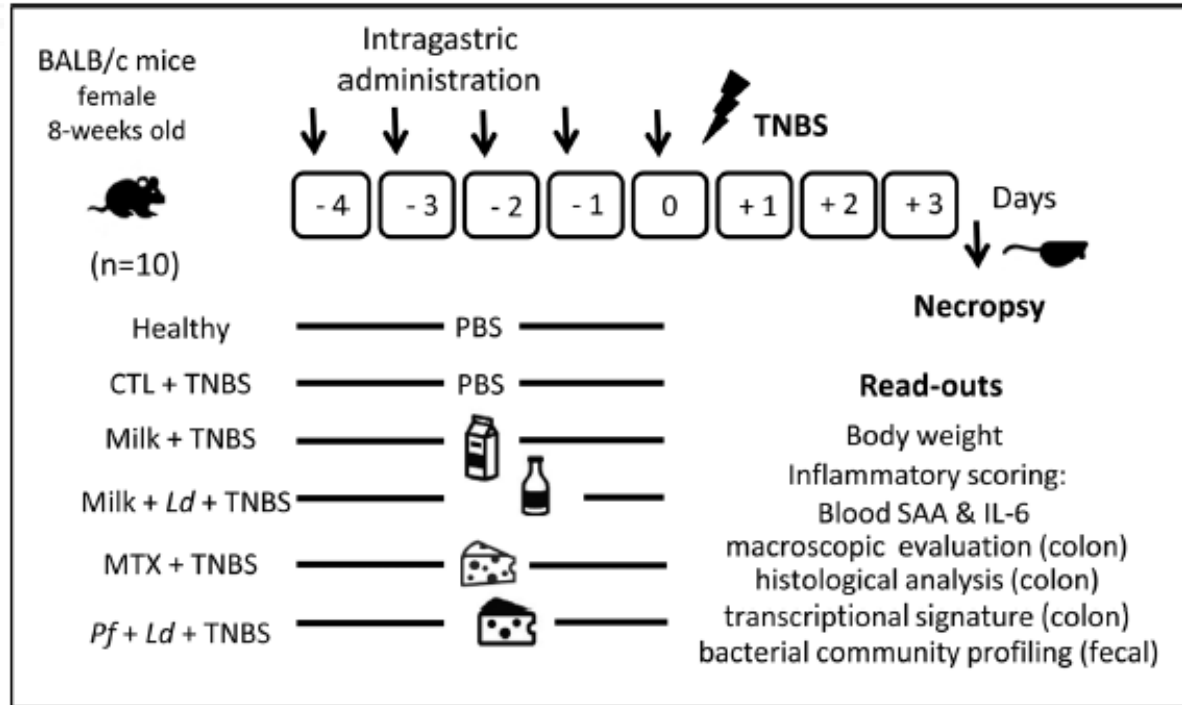
Key proteins are expressed in the cheese



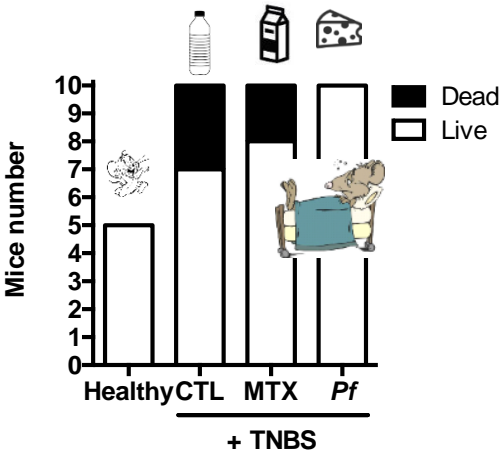
Description	Function	Gene	log(E value)	Peptides	PAI	Group	Sub-group
Surface layer protein B	Cell wall	slpB	-569,10	81	77,3	1	1.1
Surface protein with SLH domain	Cell wall	slpE	-271,21	40	21,5	1	1.2
Surface layer protein A	Cell wall	slpA	-7,48	3	0,6	1	1.3
Internaline A	Miscellaneous	inIA	-838,69	103	26,9	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



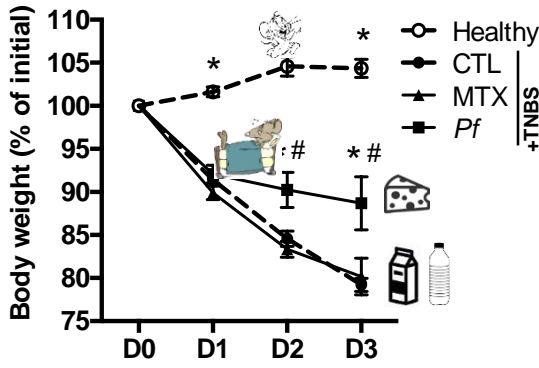
Experimental design of the animal trial



Cheese protects at the organism level



Survival

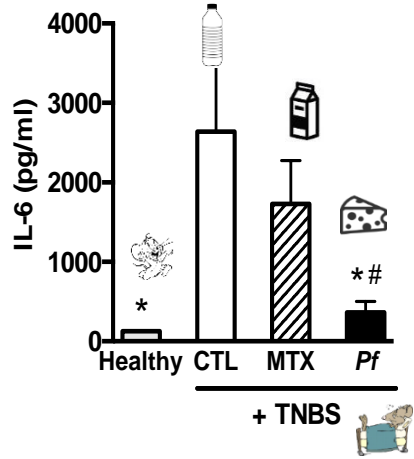


Body weight

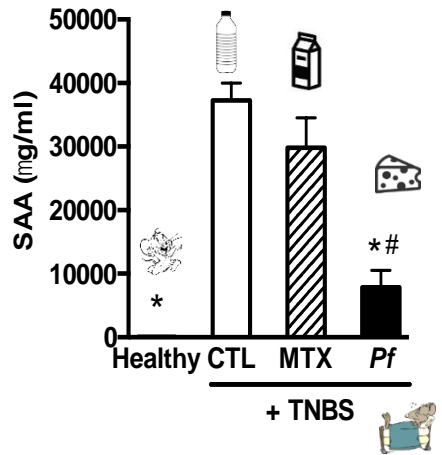
Cheese protects at the systemic level



Blood inflammation markers

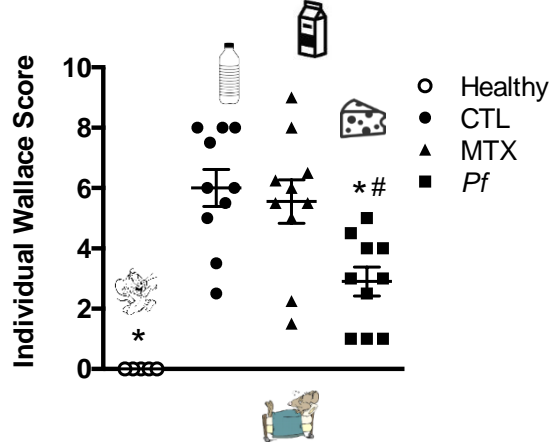
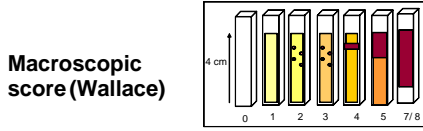
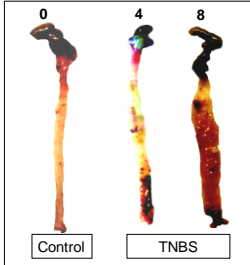
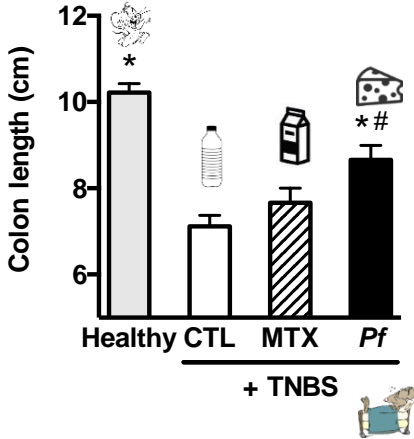


Interleukin 6

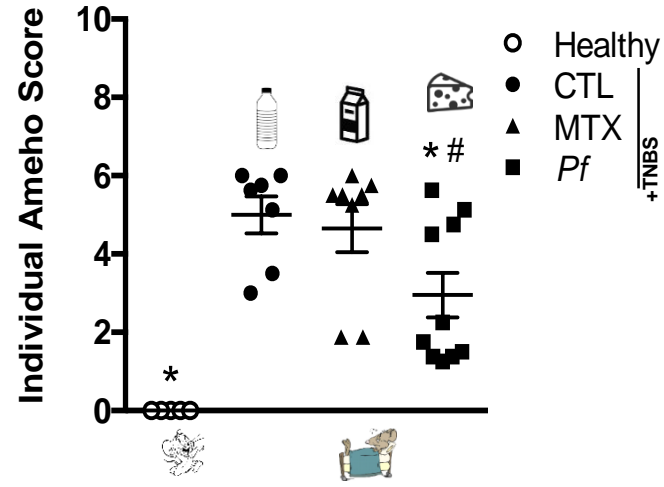
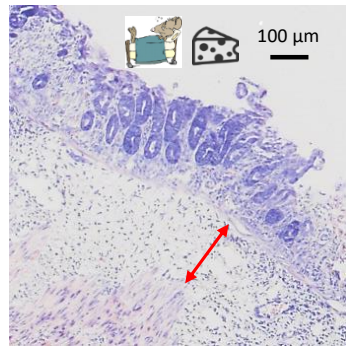
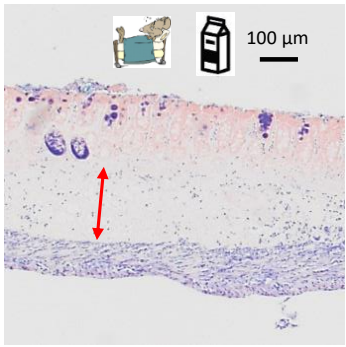
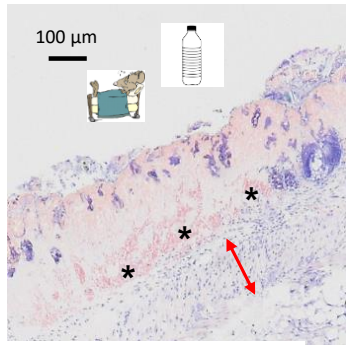
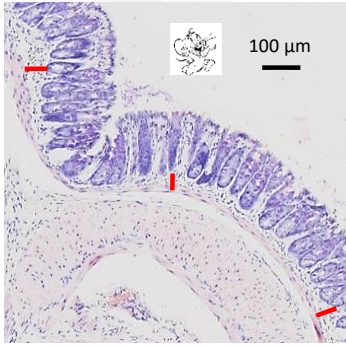


Serum Amyloid A

Cheese protects at organ level



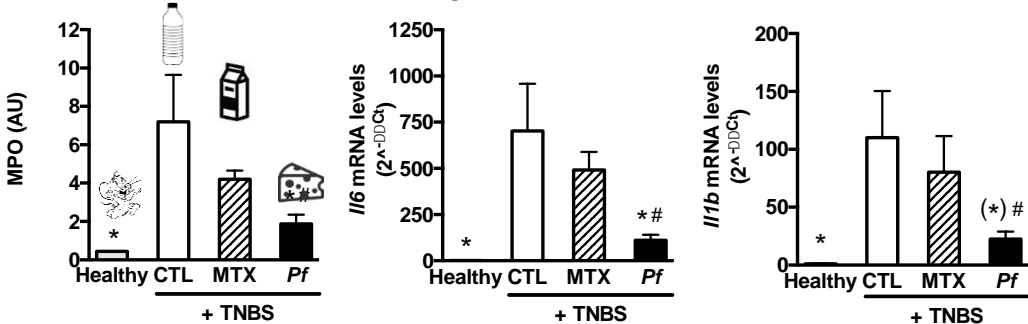
Cheese protects at the tissue level



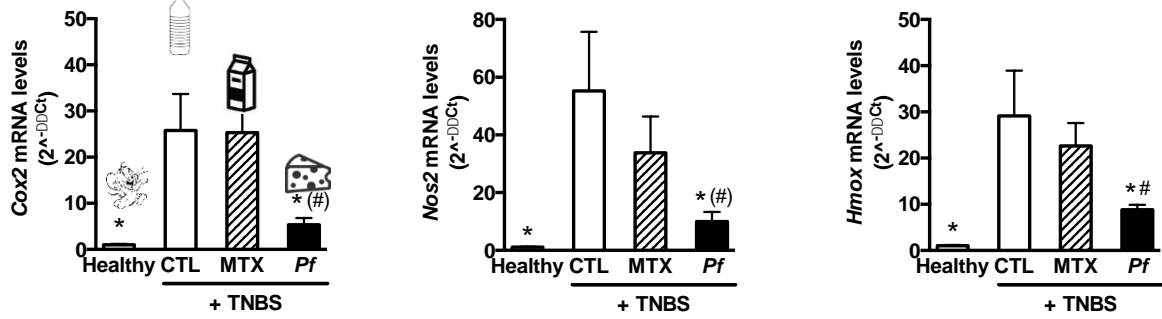
5

Cheese protects at the tissue level

Colonic inflammatory markers



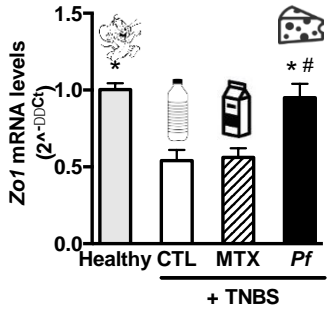
Colonic oxidative stress markers



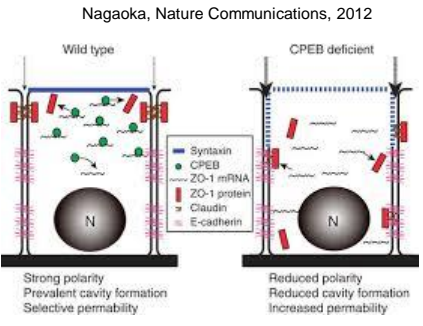
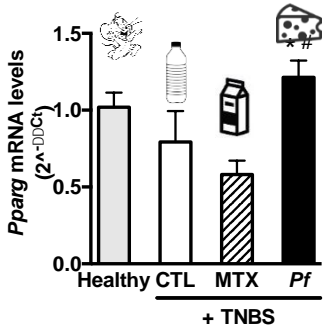
* p<0.05: vs CTL
p<0.05: vs MTX

Cheese protects at the cell level

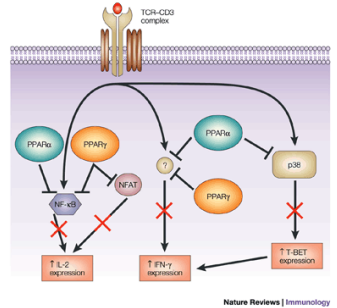
Colonic epithelial cell markers



* 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^{a,b,1}, Anna Shevchenko^{c,1}, Andrea Knaust^c, Idelisi Abuduresule^d, Wenying Li^d,
Xingjun Hu^d, Changsui Wang^{a,*}, Andrej Shevchenko^{c,*}

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 ^a	Organism ^b	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>Naumovozyima 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^{10} per day!

A huge variety of bacteria



Thank
you for
your
attention

Don't ask
tough
questions



Houem Rabah

Fillipe do Carmo

Song Huang

Floriane Gaucher

