25 years of research on dairy Propionibacteria: fighting cancer and inflammation

Gwénaël Jan

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https://www6.rennes.inrae.fr/stlo

STLO
Science & technologie du lait & de l'œuf

Milk & Egg Science and Technology

~25 Docs and Post-Docs / year

~15 Students (Master)

16 members of R&D dept (private sector)

INRAE

Probiotic diary Propionibacteria

G. Jan

Qualiment
Réseau de recherche pour l'innovation alimentaire

Structuration and coordination of international partnership

LIA BACTINFLAM (Federal Univeristy of Minas Gerais, Brésil)

2000-2020
20 ANS DE COLLABORATION
100 PUBLICATIONS

LIA FOODPRINT (Soochow University, China)

10 ANS DE COLLABORATION
30 PUBLICATIONS
12 PUBLICATIONS LIA (2 ANS)

2RI INFOGEST, STLO

- **International visibility**
- **Design of collaborative projects**
- **1/3 of foreign PhD students** (15 Brazilians, 3 Chineses)

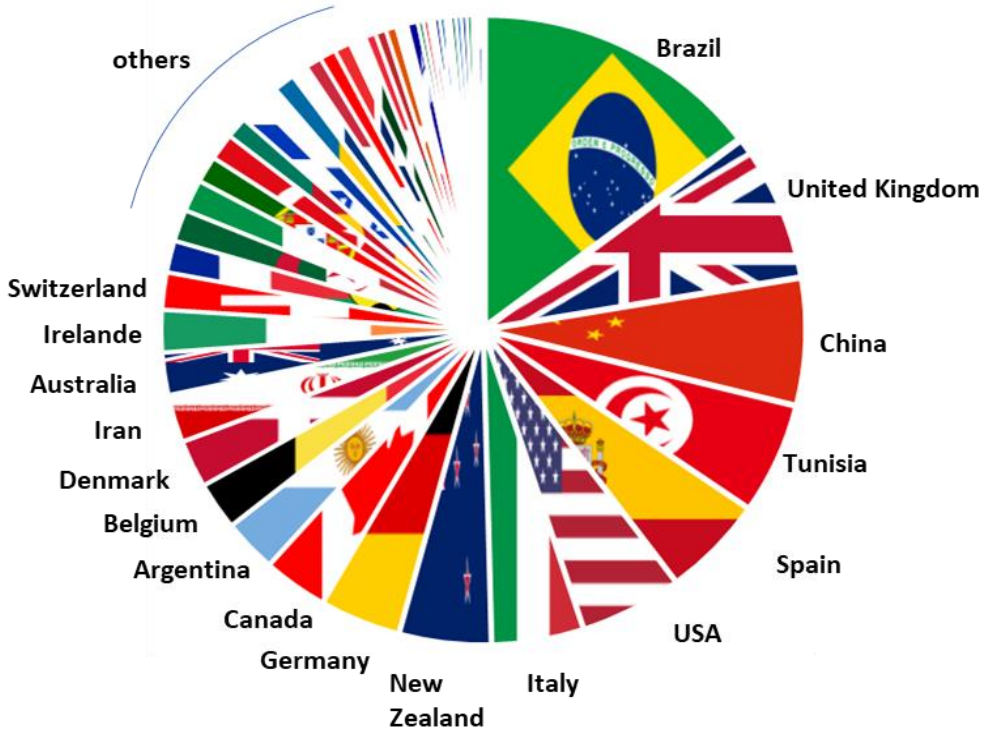
Scientific Production

394 Peer-Reviewed Papers / 5 years (+30%)

75% in Q1

2,5 PRP/Res./y.

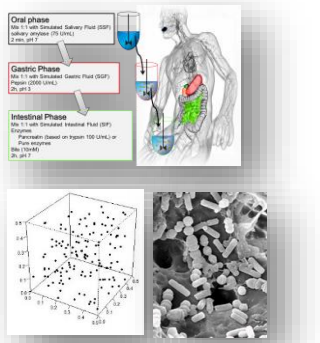
1st lab of the Bretagne Normandy INRAE Centre in PRP / Res.

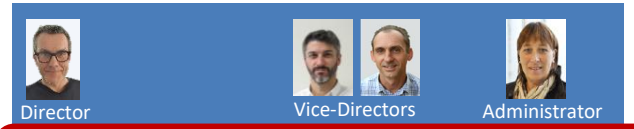


51% of papers co-authored with foreign collaborators

(Top1 et Top3 Brazil and China > IAL)

Organization

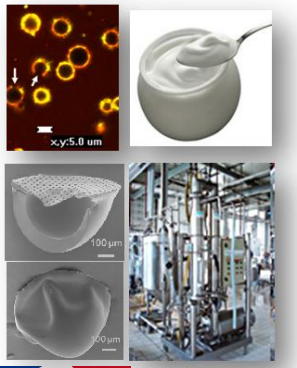




Optimisation of infant formulas

Animal-plant mix

SAPHIRE
Administration,
Informatics & Infrastructure, Doc & Com



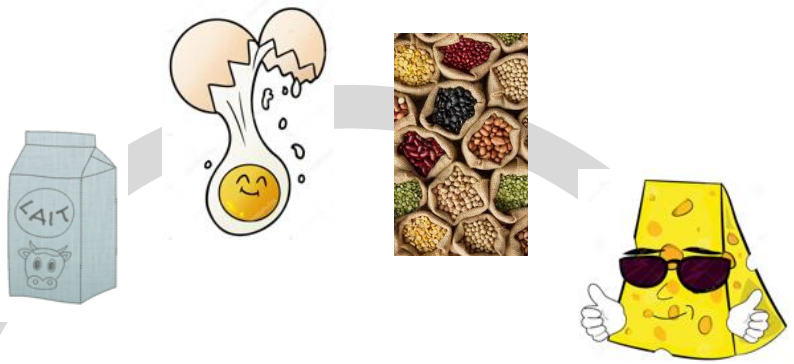
MICROBIO

Food quality & safety

Develop safe, healthy, sustainable and hedonic fermented foods

Food spoilage

Reduce health risks and food losses and waste



Characterize interactions between bacteria/environment

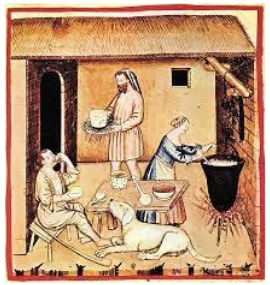
Animal health

Offer alternatives to antibiotic treatments (probiotics)

Health & wellbeing

Develop functional food against modern lifestyle diseases or promoting health in infants

Emmental and other cheeses with holes: a long story



Flavour

Sweet

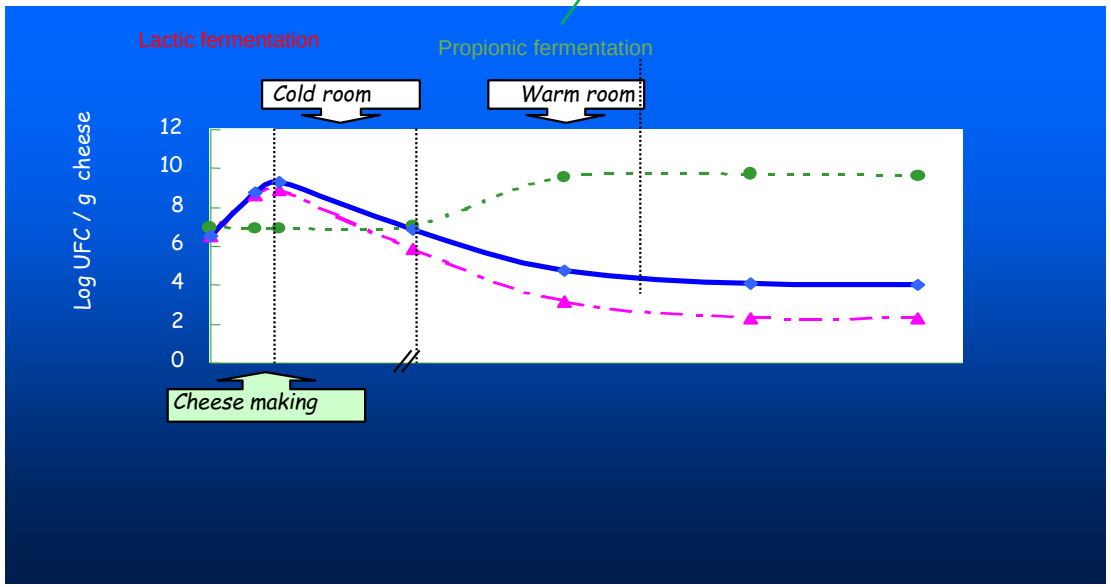
Sour

Picante

Esters ← Propionate

Acetate

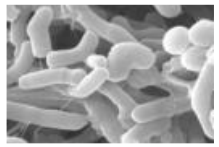
CO_2



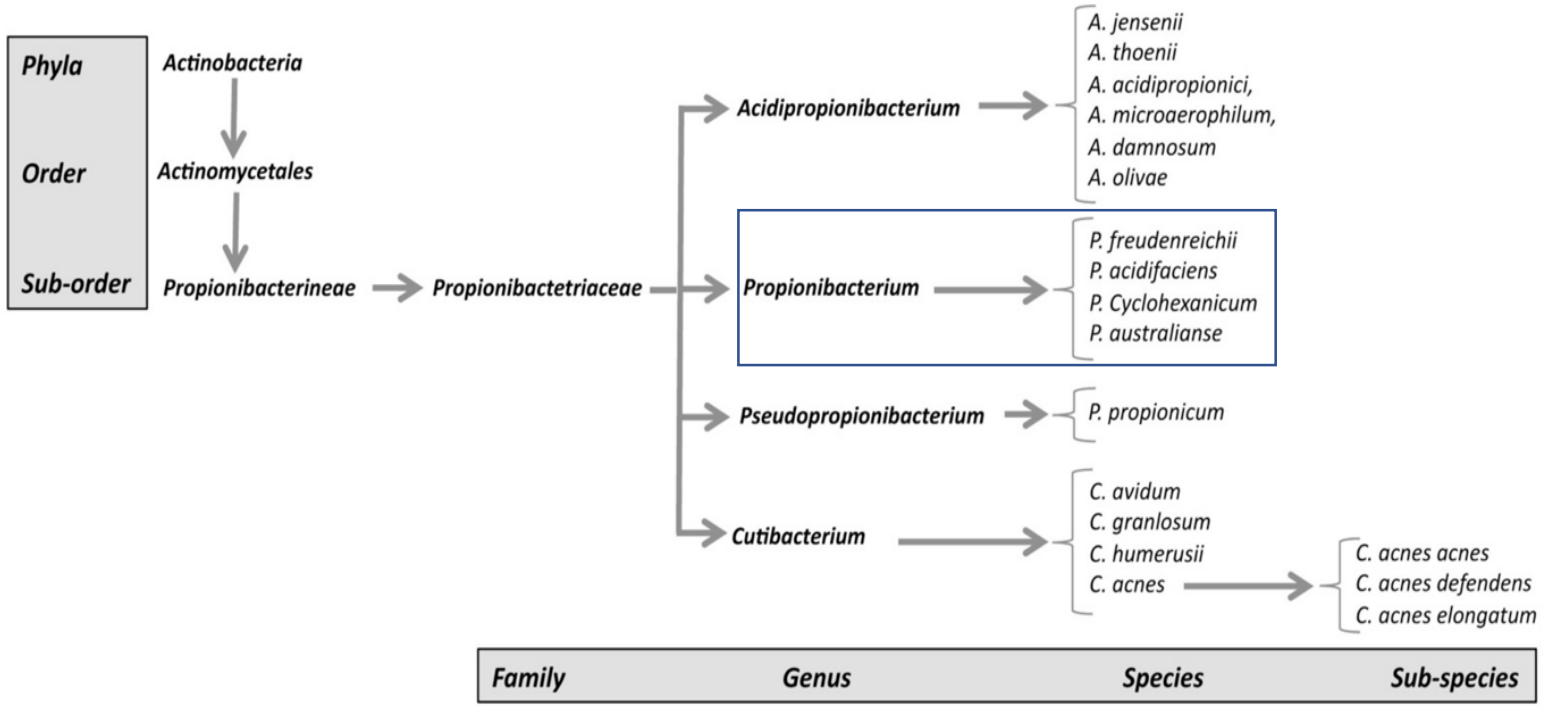
➔ **The Fitz equation (1878)**

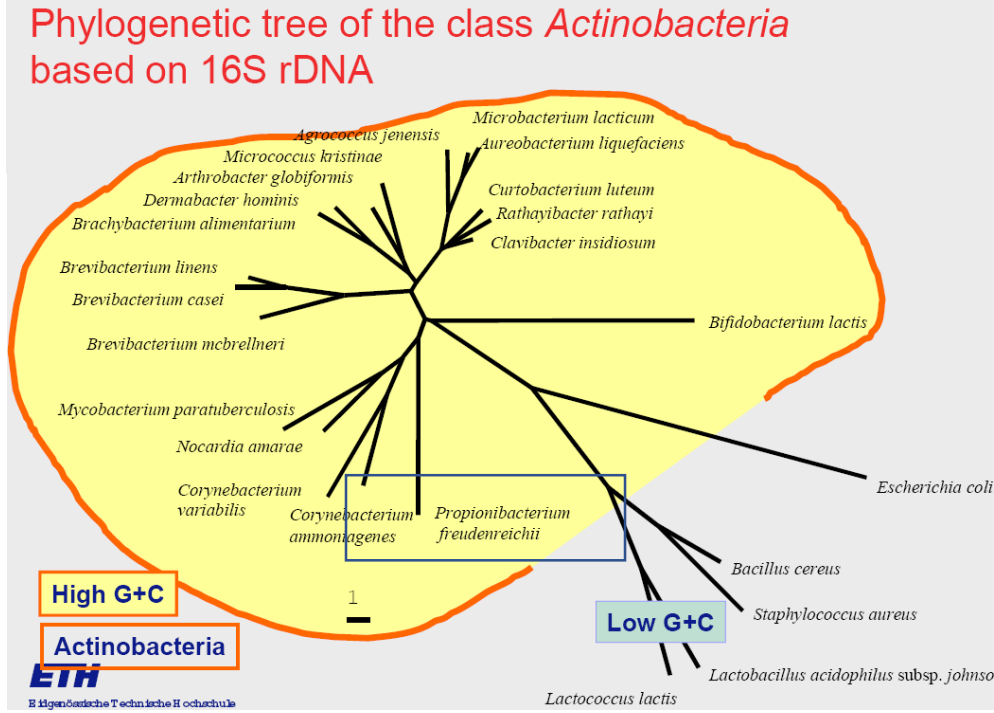
3 Lactate ⟶ 2 propionate + 1 acetate + 1CO_2

➔ **The isolation of dairy propionibacteria by von Freudenreich and Orla-Jensen (1907)**



Propionibacteria, in a few words





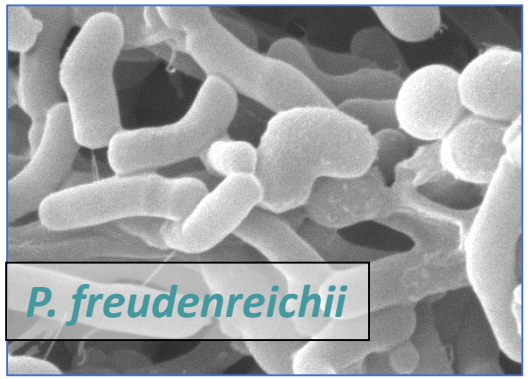
Gram +

pleiomorphic

anaerobic to microaérophilic

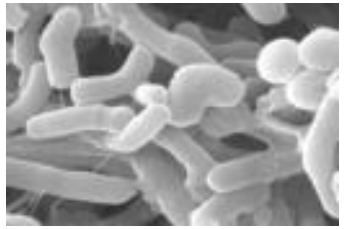
non motiles et non sporulated

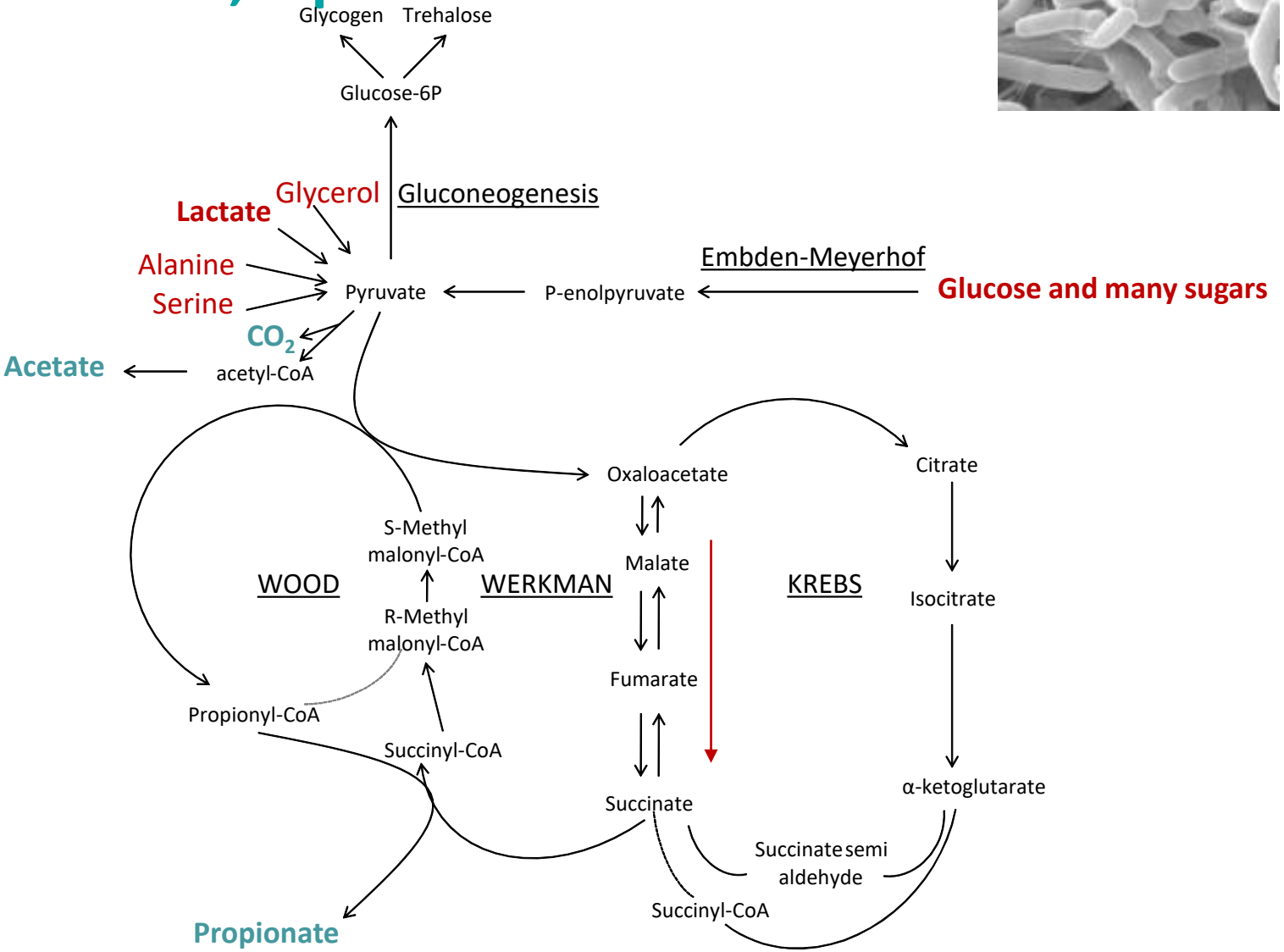
GC Rich : 67%



Generation time : 5 to 6 hours !

Propionibacteria, a peculiar metabolism





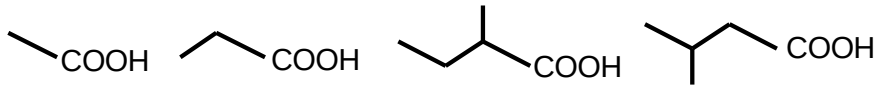
P. freudenreichii is a sort of Swiss army knife

➔Vitamins B9, B12

➔Probiotics

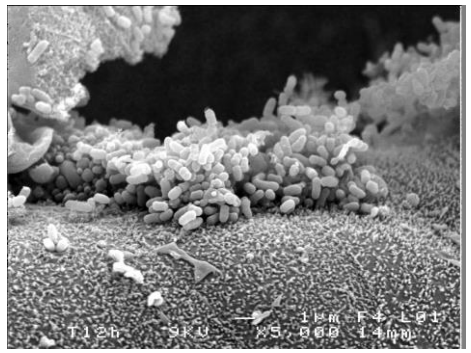
➔ Bio-control

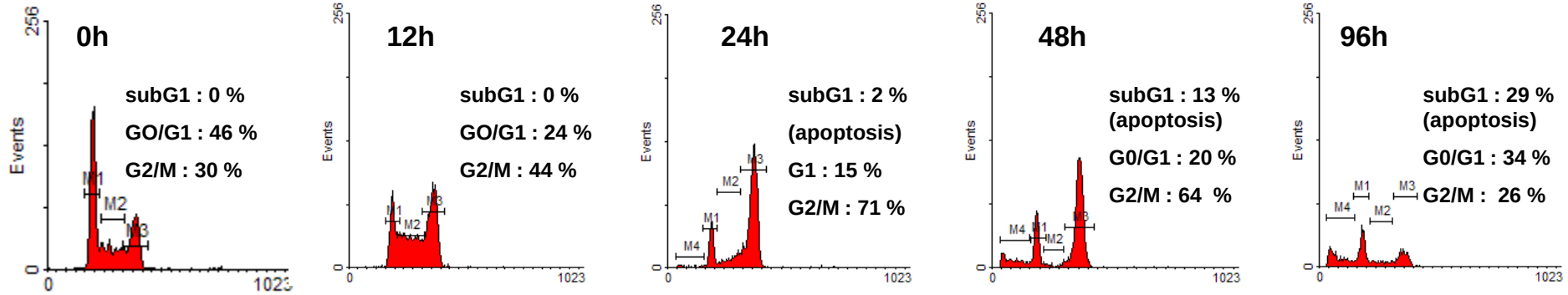
➔ Ripening starter



Short Chain Fatty Acids (SCFA): Known effects on health, differentiation and on apoptosis

Propionibateria metabolites: impact on cell proliferation (*in vitro*)





Caco-2 colon cancer cells and
Propionibacterium freudenreichii

➔ **Decrease proliferation and induce cell death**

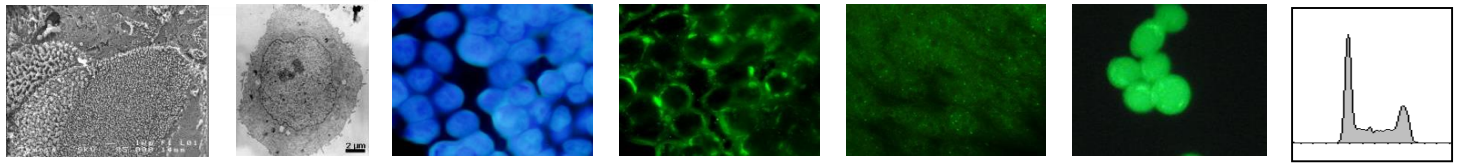
Catherine Brenner
Directrice du laboratoire METSY

Marie-Thérèse Dimanche-Boitrel, INSERM, IRSET

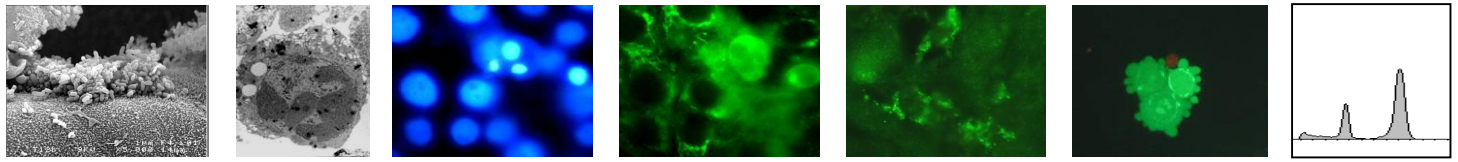
Kills cells in co-culture

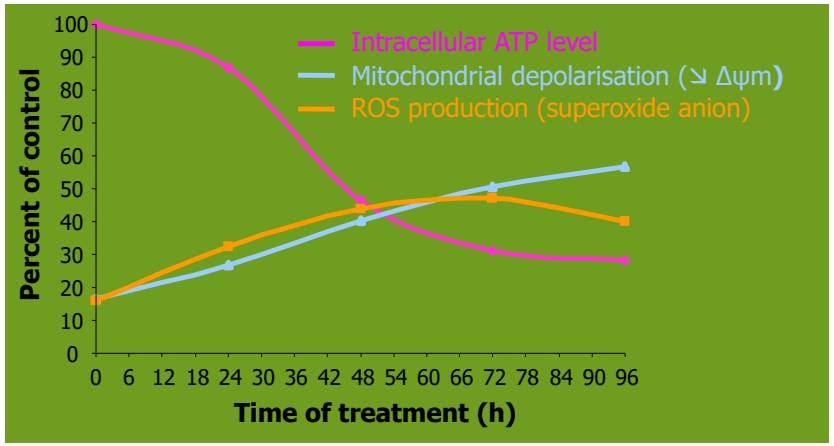
By apoptosis

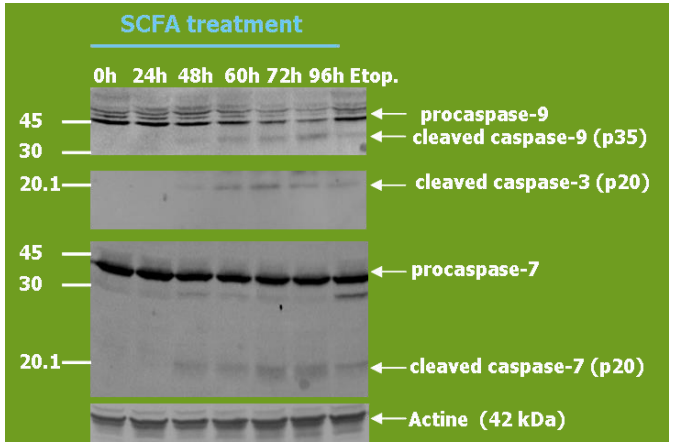
➔ Caco2 cells



➔ In co-culture





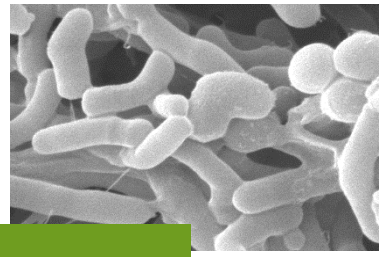


➔ It is apoptosis

➔ Similar results with the SCFA acetate & propionate

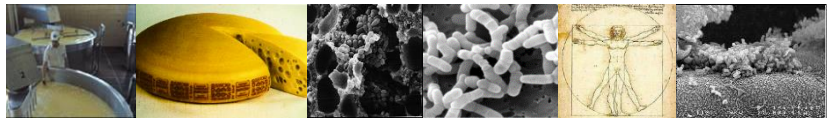
Kills cells in co-culture

By apoptosis



OK, but would this happen with a fermented dairy product?

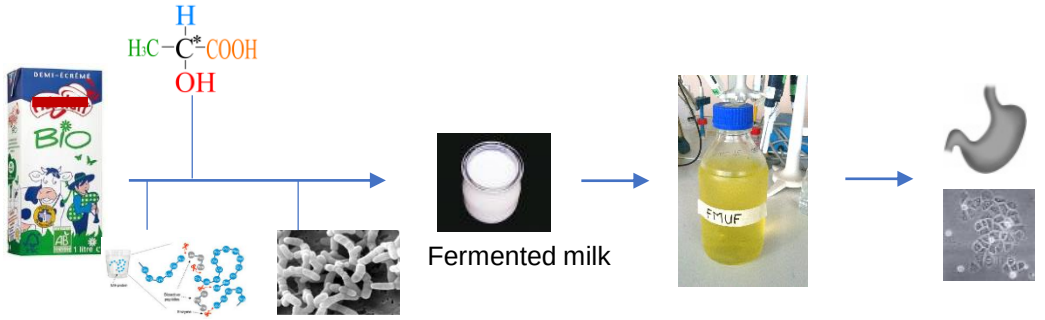
CONTENTS

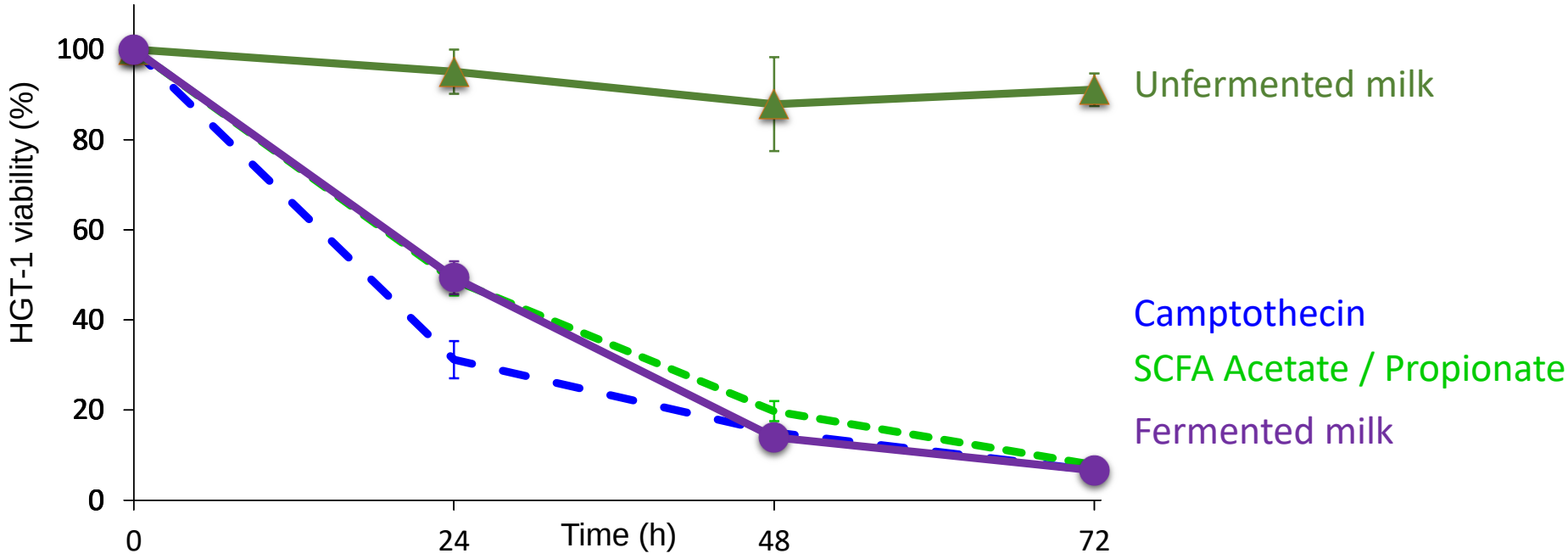


- *Propionibacterium freudenreichii* induces apoptosis in co-culture *in vitro*.
- Milk fermented by *P. freudenreichii* induces apoptosis of gastric and colon cancer cells *in vitro*.

A new fermented milk

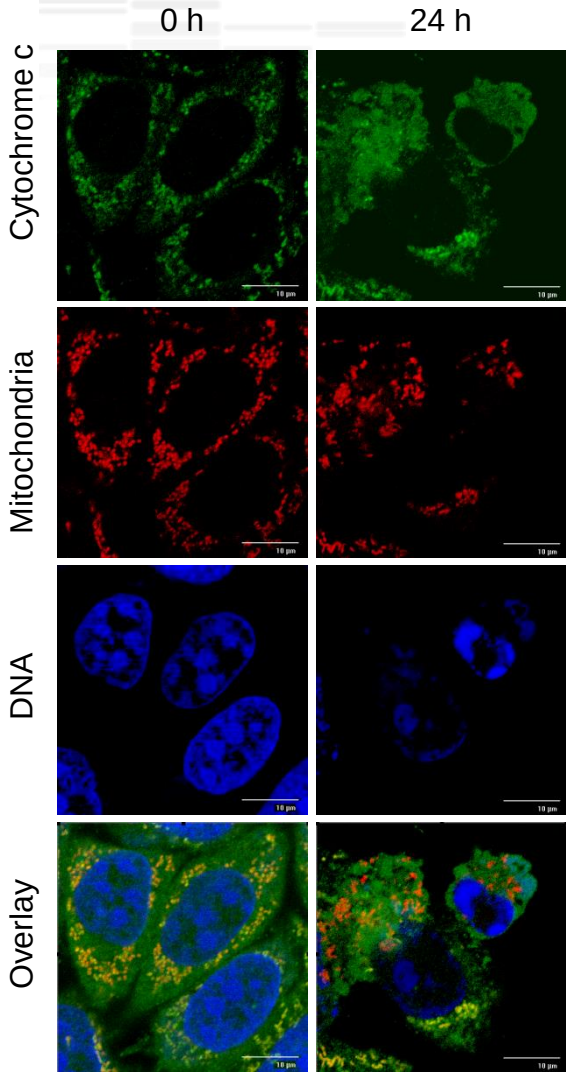
Effect on gastric cancer cells





A new fermented milk

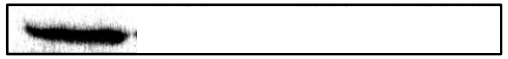
Effect on gastric cancer cells mitochondria



Western Blot /cytoplasm

0 h

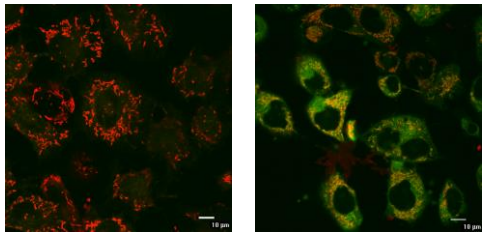
Cytochrome c



Hsc 70

↳ **Cytochrome c leakage**

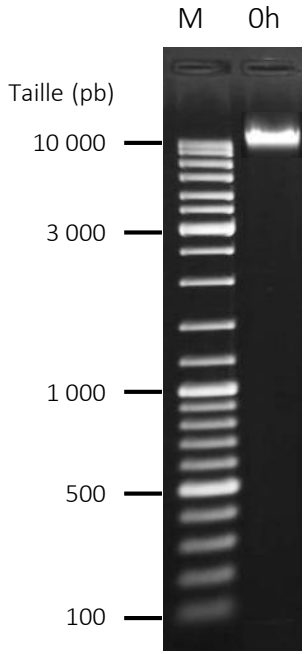
Control-JC1 | Fermented UF ½ (24 h)



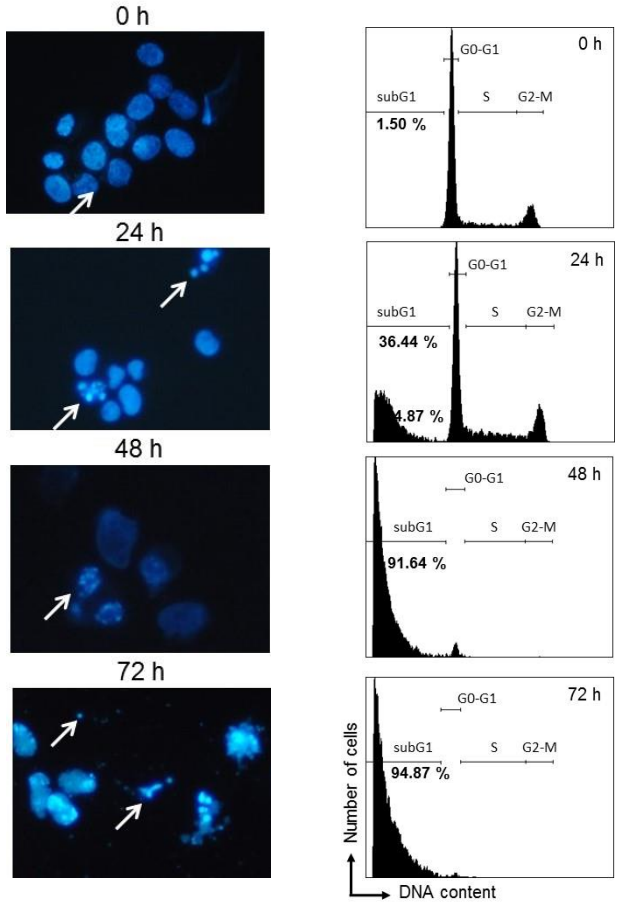
↳ **Mitochondria depolarisation**

A new fermented milk

Effect on gastric cancer cells chromatin



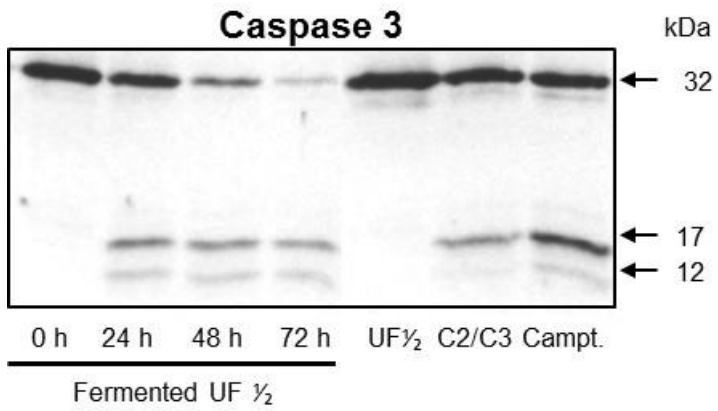
↳ **DNA fragmentation**

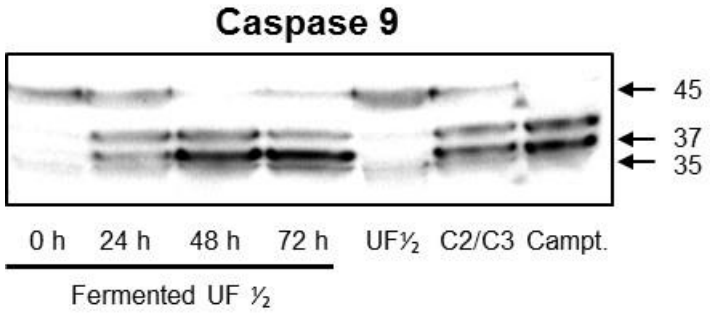


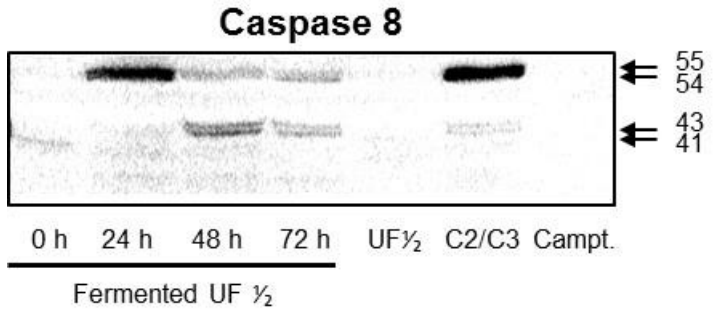
↳ **Apoptotic bodies**

A new fermented milk

Effect on gastric cancer cells caspases







↳ **Caspases activation**

A new fermented milk

Effect on camptothecin efficacy

OK, but do propionibacteria reach the colon alive?

↳ **An additive effect**

↳ **Similar results on HT29 cells**

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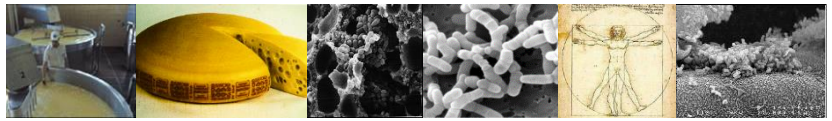

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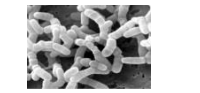
- Milk fermented by *P. freudenreichii* induces apoptosis of gastric and colon cancer cells *in vitro*.
- **Milk fermented by *P. freudenreichii* bring live and active bacteria to the colon *in vivo*.**

A new fermented milk

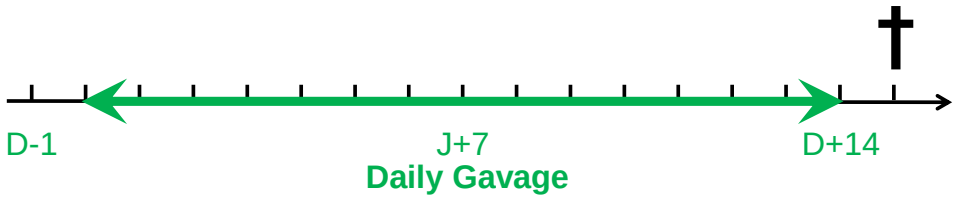
Allows metabolic activity *in vivo*

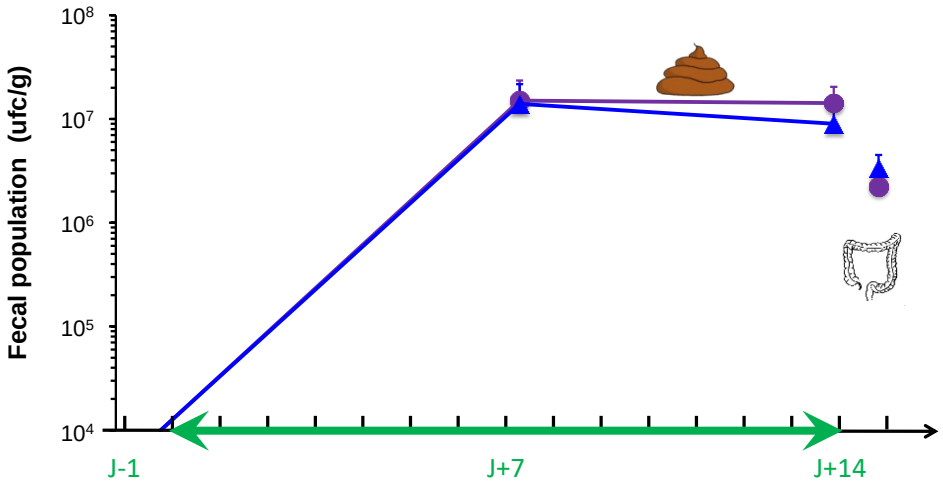


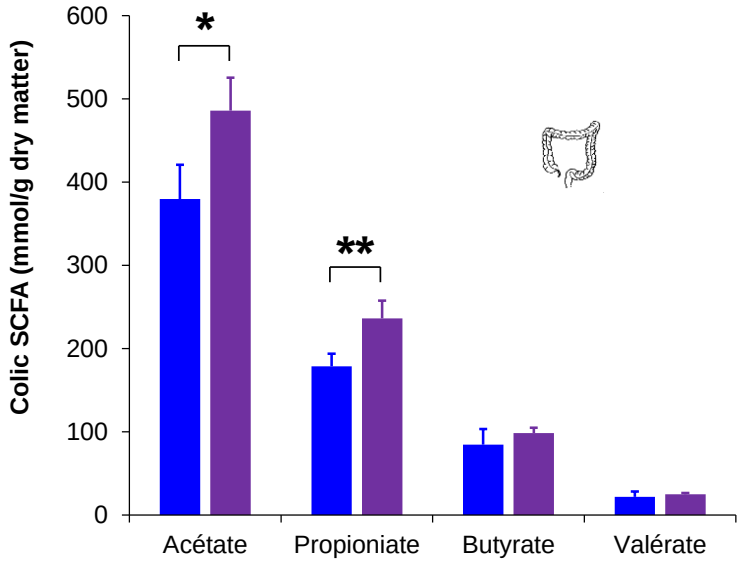
P. freudenreichii

Freeze-dried

Fermented milk







↳ **Metabolic activity *in situ* depends on food delivery vehicle**

A new fermented milk

Has an effect on animal physiology *in vivo*

Unfermen

Fermented

Plasma Haptoglobin

Colon explants cytokines

OK, but what is the impact of propionibacterial metabolites on colon carcinogenesis *in vivo*?

↳ **Effect on gut physiology**

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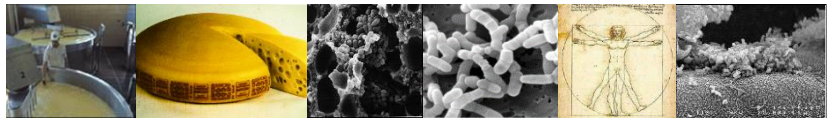

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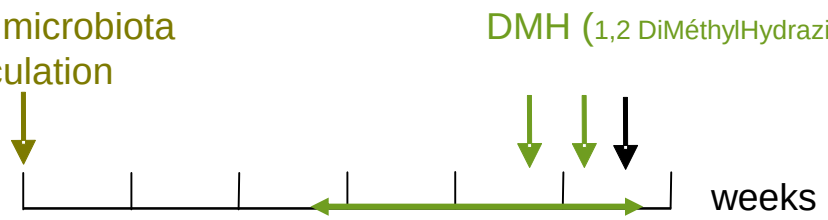
- Milk fermented by *P. freudenreichii* bring live and active bacteria to the colon *in vivo*.
- Ingested *P. freudenreichii* favours apoptotic depletion of damaged colonocytes in the context of carcinogenesis *in vivo*.

Human microbiota associated (HMA) rats

With carcinogenesis : Dimethylhydrazine (DMH)

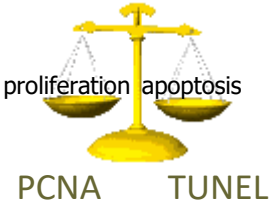
4 batches of 6 axenic rats



Daily gavage, 10^9 / day

Apoptosis : TUNEL labeling

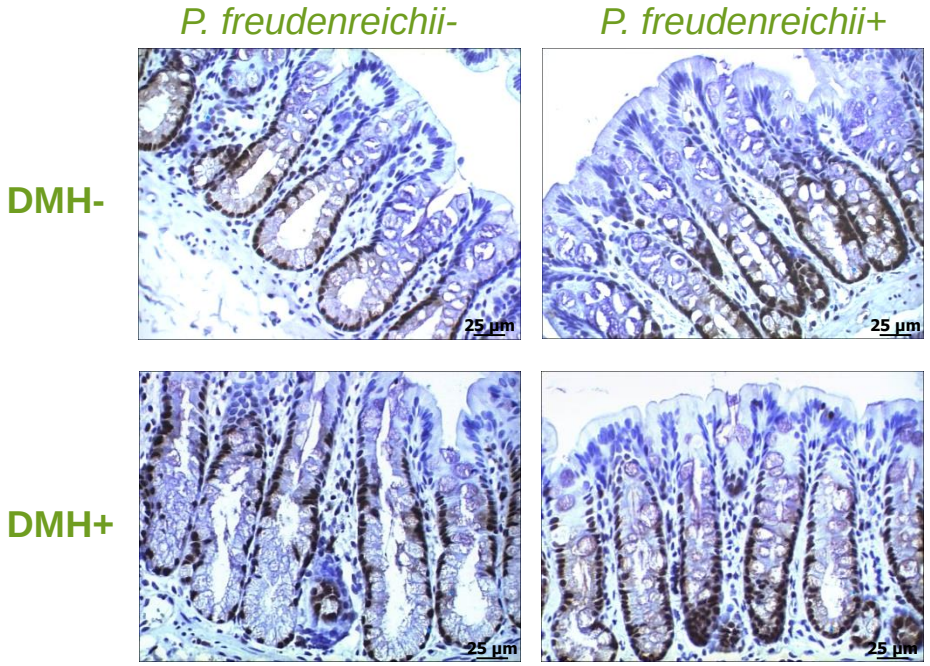
Proliferation : PCNA immunolabeling

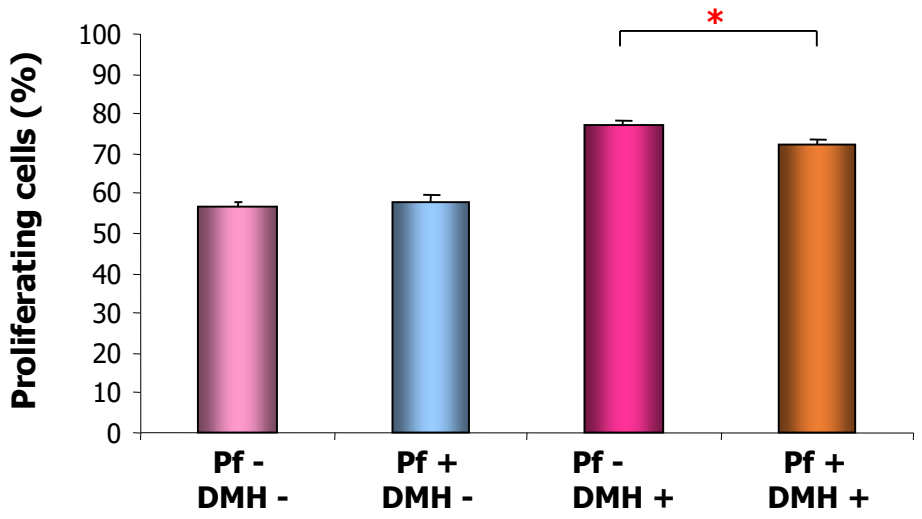


Sylvie Rabot,
INRAE, Micalis

Human microbiota associated (HMA) rats

PCNA labelling : proliferation

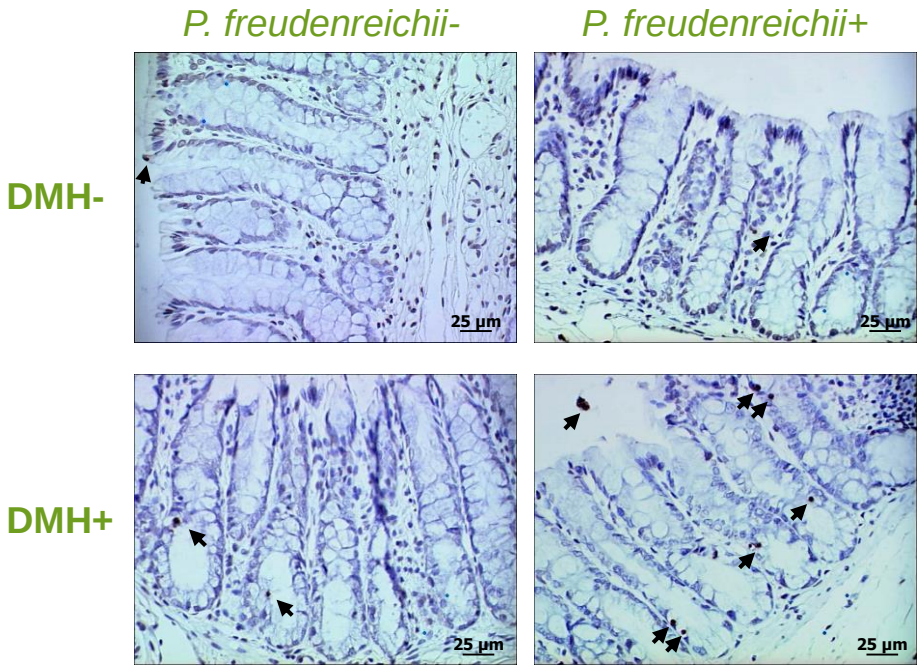


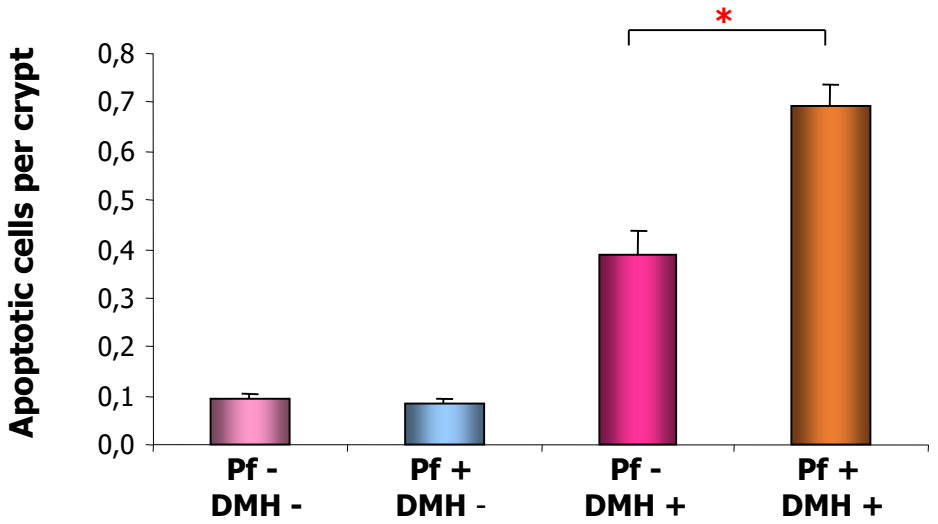


- **No effect of *P. freudenreichii* on cell proliferation in control rats**
- ***P. freudenreichii* little effect on proliferation in mutagenized rats**

Human microbiota associated (HMA)rats

TUNEL labelling : apoptosis

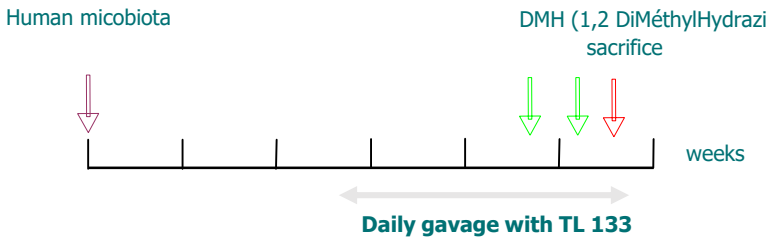


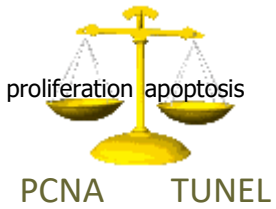


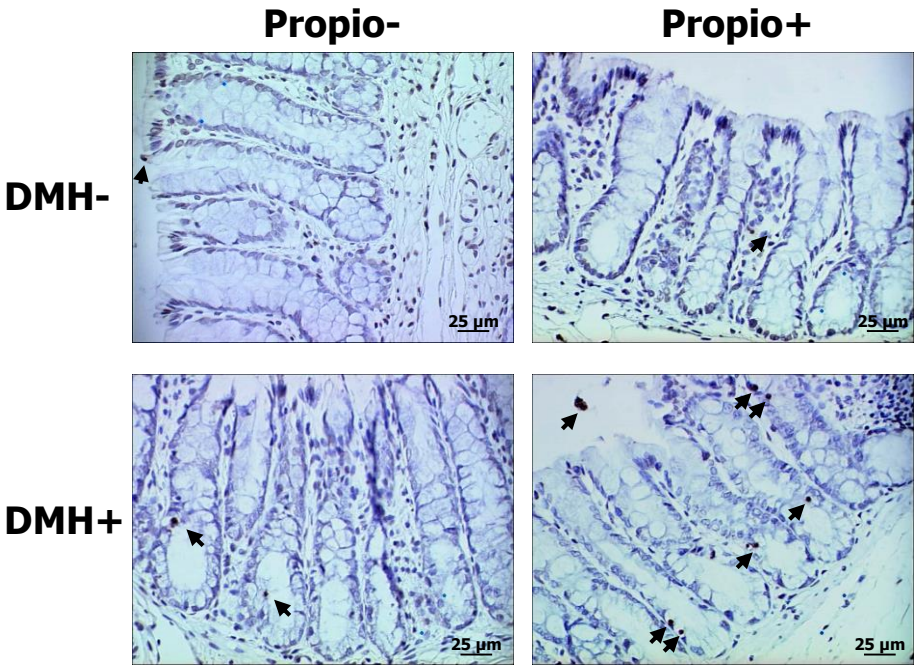
- No effect of *P. freudenreichii* on apoptosis in control rats
- *P. freudenreichii* increases apoptosis in mutagenized rats

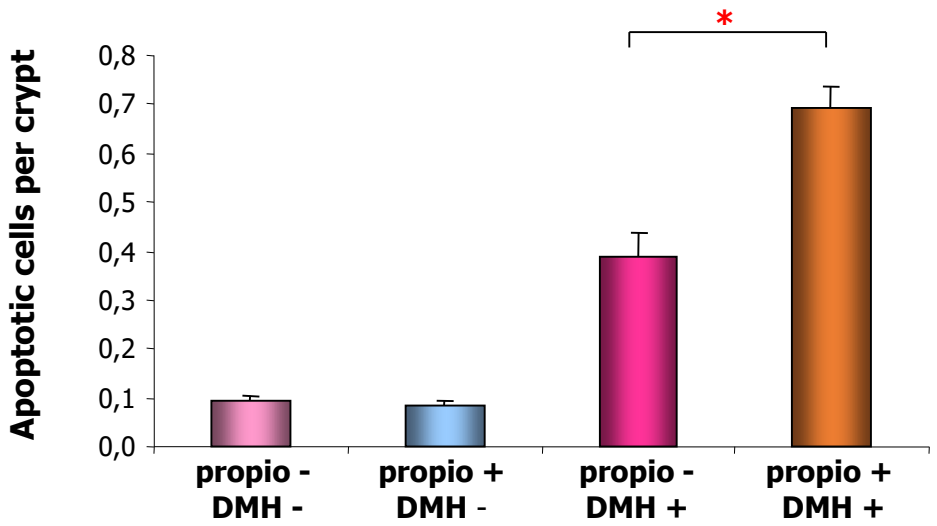
Propionibateria: increasing apoptosis of cancer cells (*in vivo*)

4 batches of 6 axenic rats









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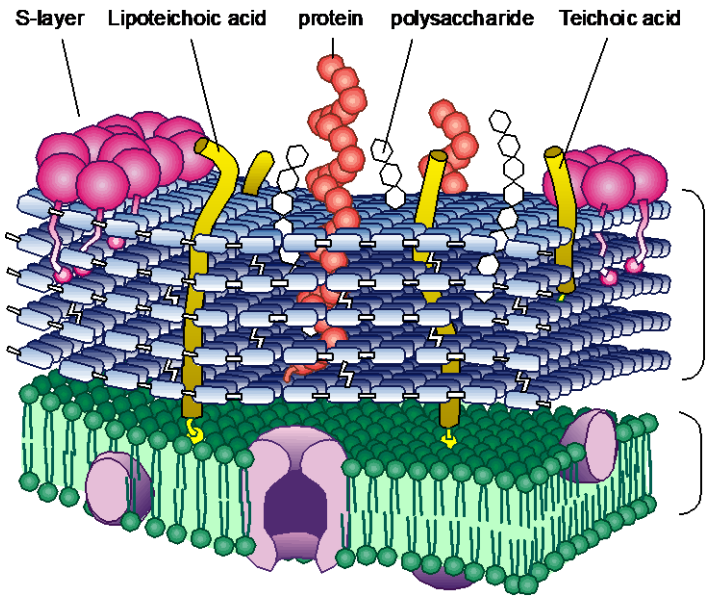
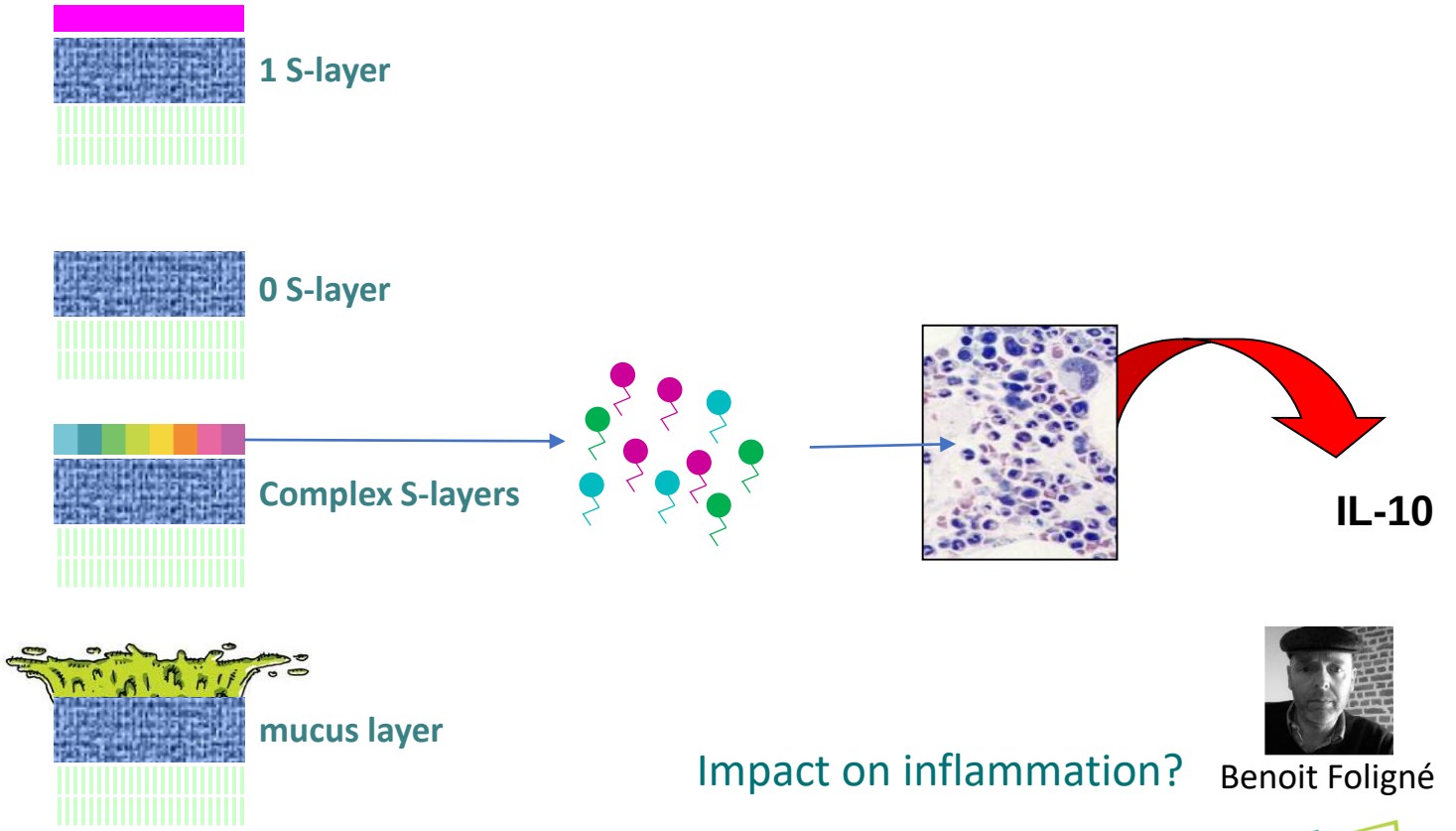
CONCLUSION

- ***Propionibacterium freudenreichii*** **induces apoptosis in co-culture *in vitro*.**
- **Milk fermented by *Propionibacterium freudenreichii* induces apoptosis of gastric and colon cancer cells *in vitro*.**
- **Milk fermented by *Propionibacterium freudenreichii* bring live and active bacteria to the colon *in vivo*.**
- **Ingested *Propionibacterium freudenreichii* favours apoptotic depletion of damaged colonocytes in the context of carcinogenesis *in vivo*.**

A food supplement to synergize prevention or treatment of digestive cancer ?

Propionibacteria, a peculiar architecture





Impact on inflammation?

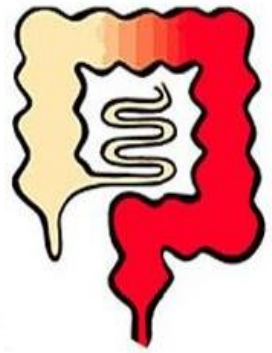
Benoit Foligné

A growing incidence of inflammatory ailments

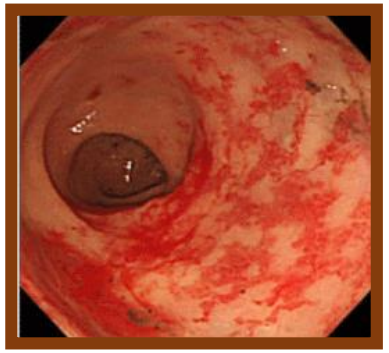
IBD

- GIT Chronic inflammation

Ulcerative
Colitis



Mucositis



Oral mucositis

GI Mucositis

5-Fluoracil (5-FU)

- Head, neck and colon cancer.

(Chang *et al*., 2012; Falvey *et al*., 2015)

A growing incidence of inflammatory ailments

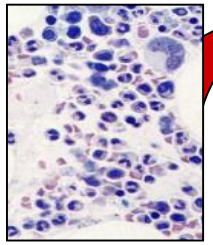
IBD

- GIT Chronic inflammation

Ulcerative
Colitis

In human immune cells: PBMCs (peripheral blood mononuclear cells)

+



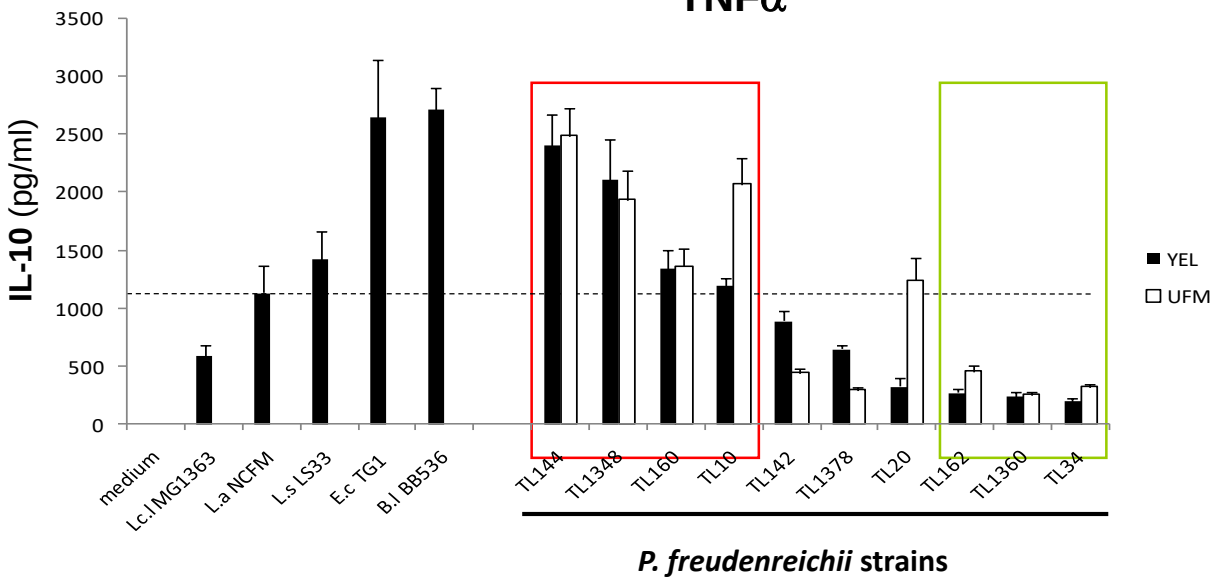
24 h

IL-10

IFNγ

IL-12

TNFα



***P. freudenreichii* strains**

Bruno Pot

Benoit Foligné

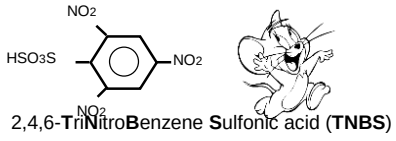
The best anti-inflammatory strains

The less anti-inflammatory strains

Probiotic diary Propionibacteria

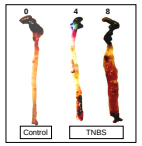
G. Jan

In an acute colitis mice model induced by TNBS

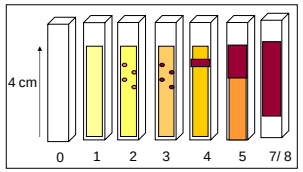


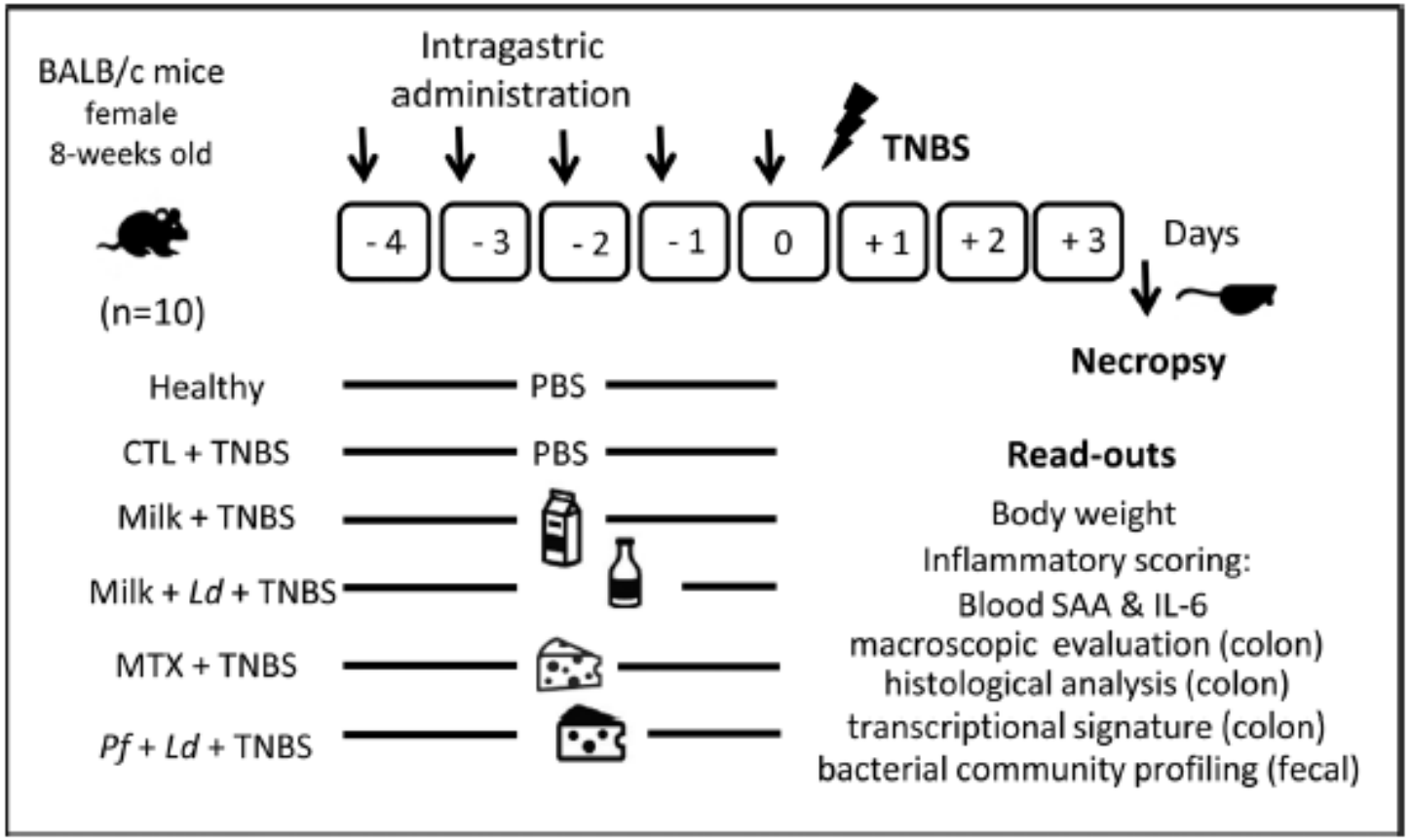
2,4,6-**Tri**Nitro**B**enzene **S**ulfonic acid (**TNBS**)

Acute colitis

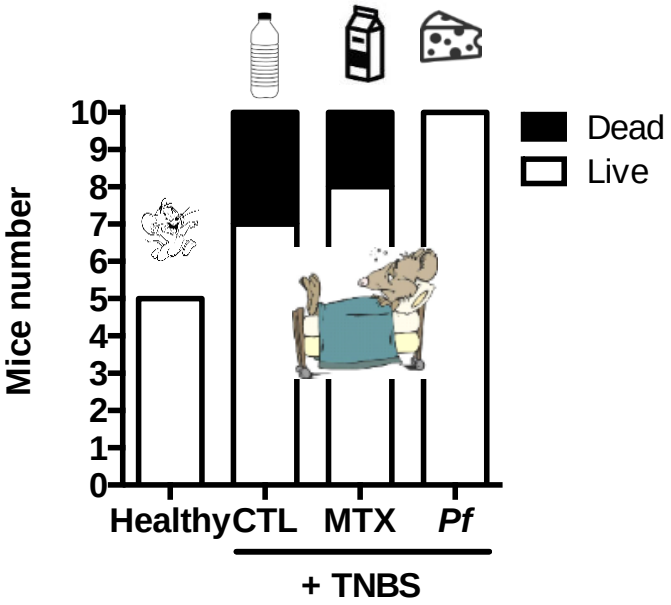


Macroscopic score (Wallace)

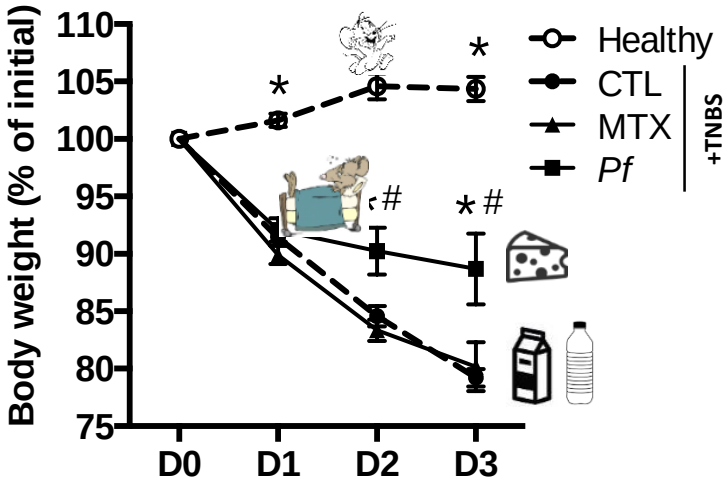




P. freudenreichii mitigates TNBS-induced colitis in mice

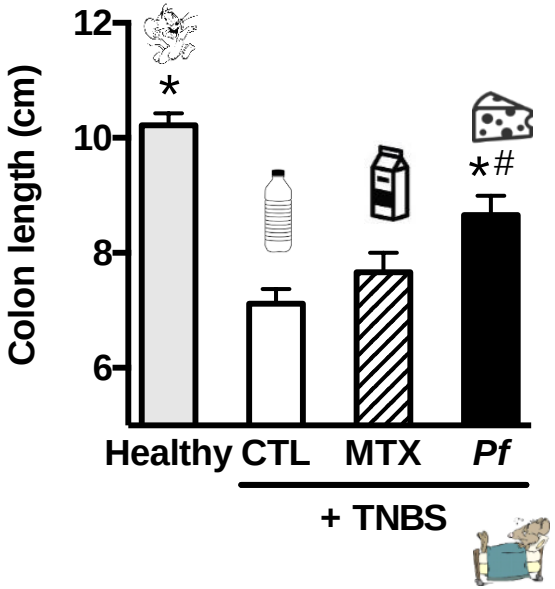


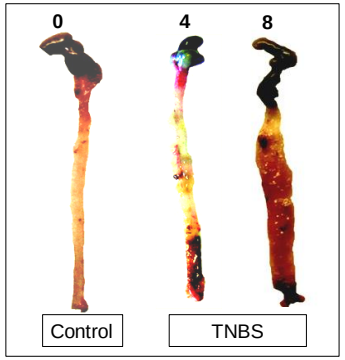
Survival



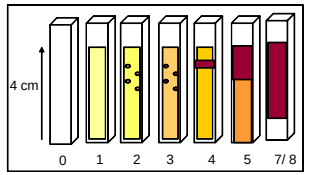
Body weight

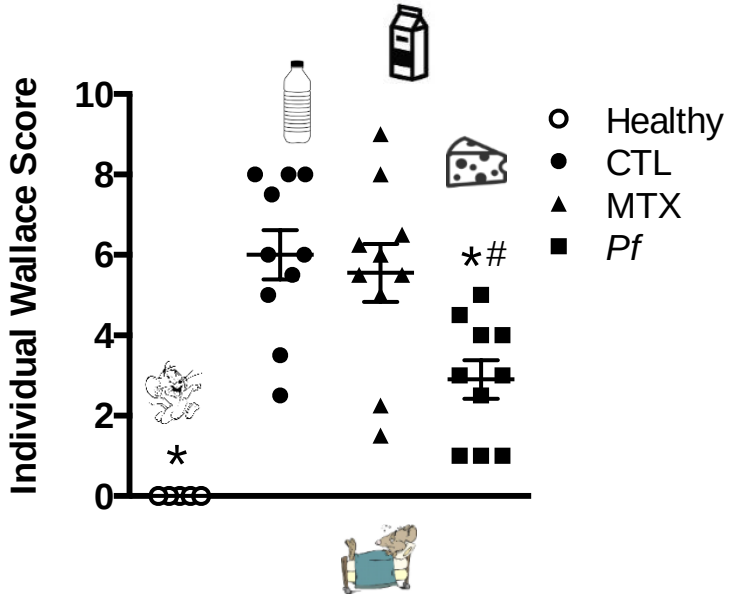
P. freudenreichii mitigates TNBS-induced colitis in mice





Macroscopic score (Wallace)





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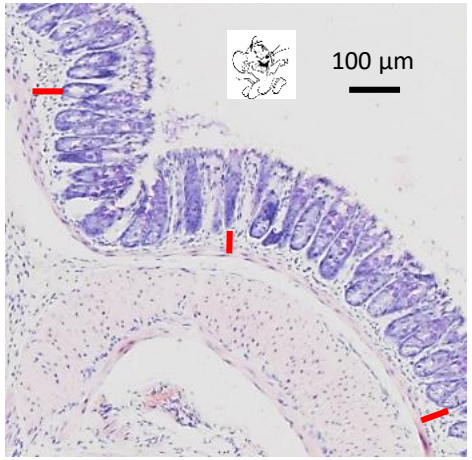
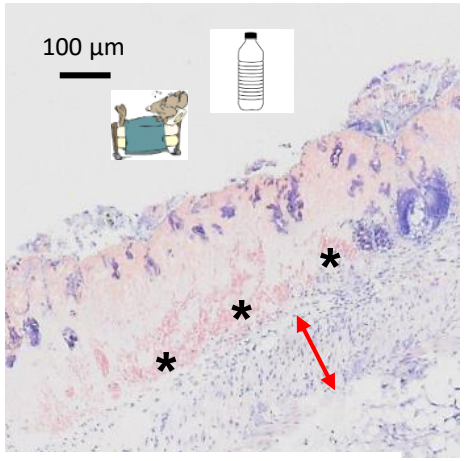
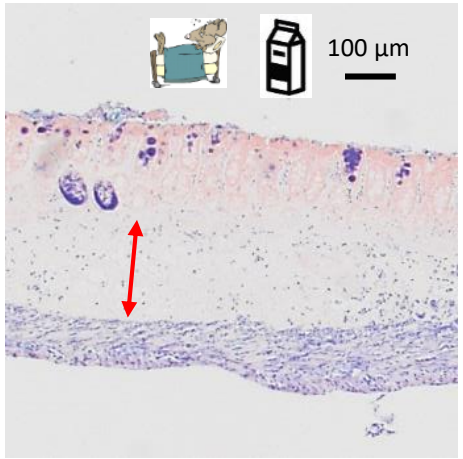
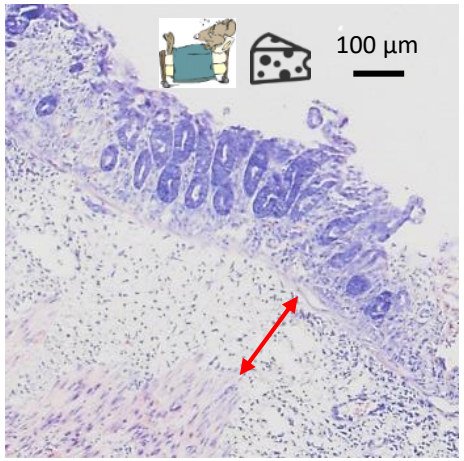
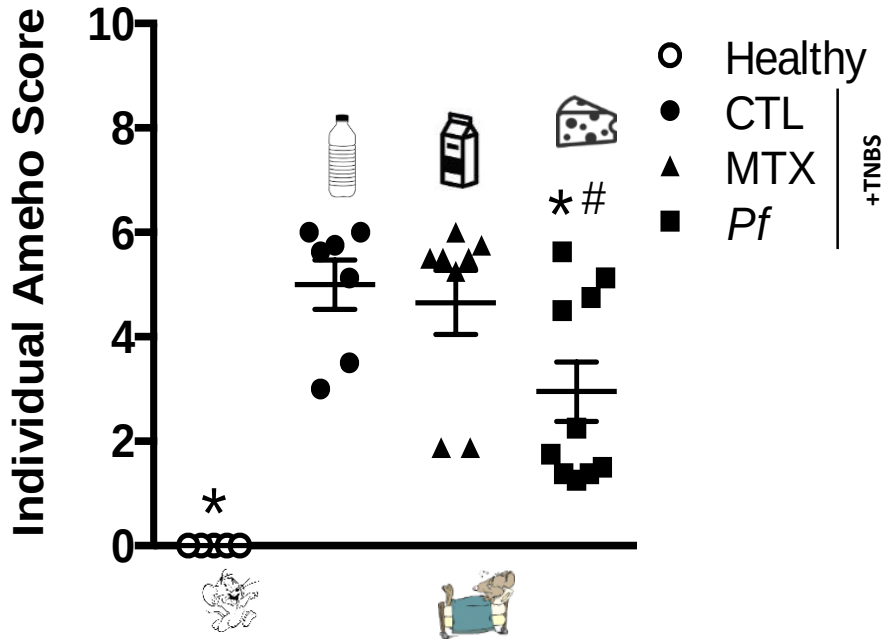


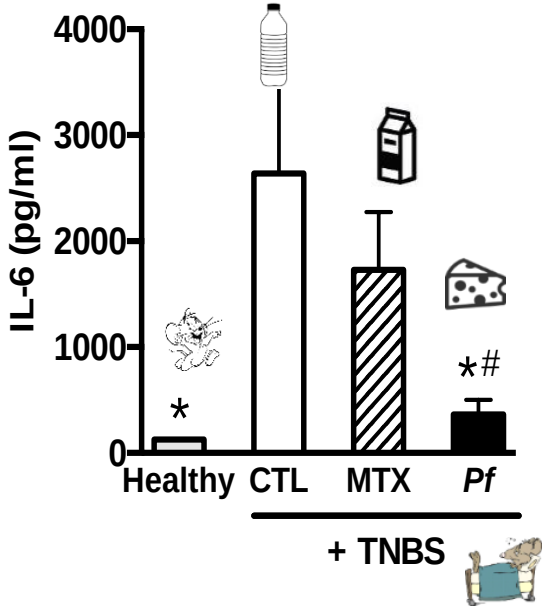




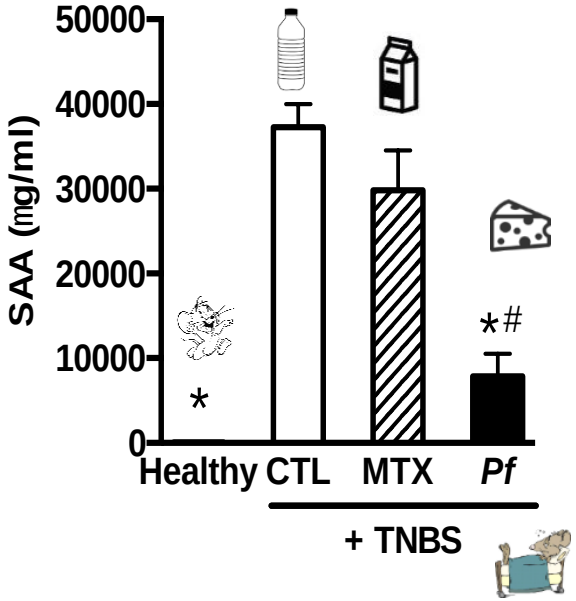




P. freudenreichii mitigates blood inflammation markers



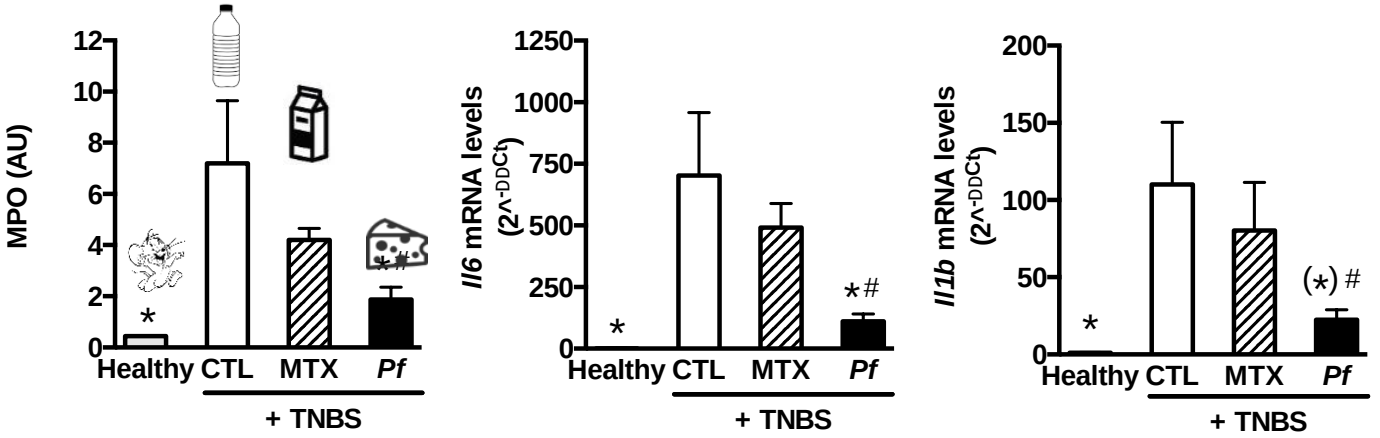
Interleukin 6

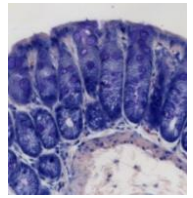


Serum Amyloid A

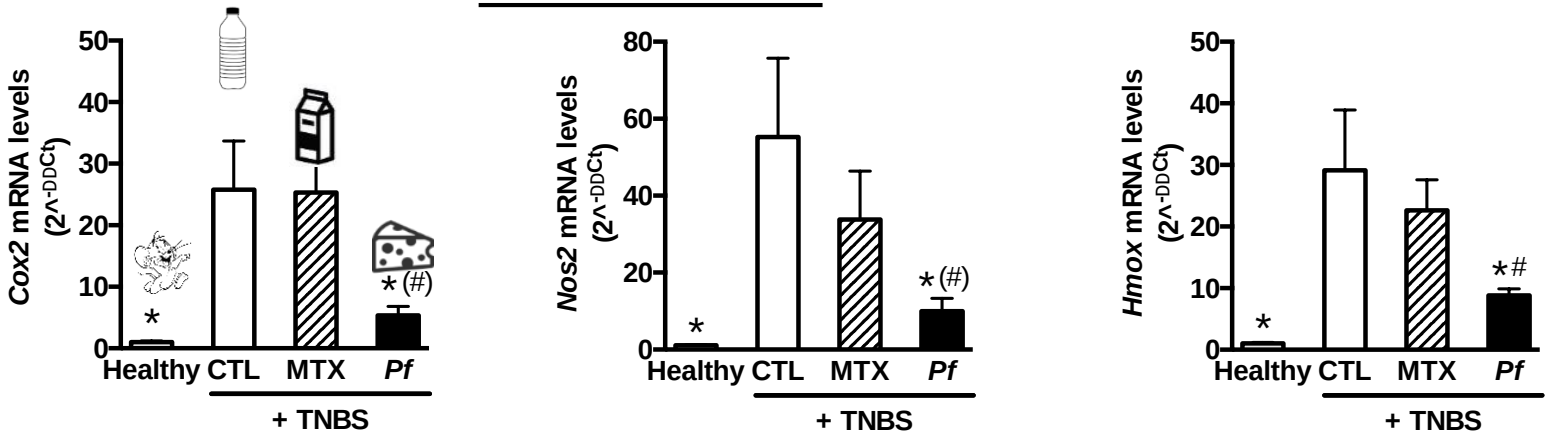
P. freudenreichii mitigates colon inflammation markers

Colonic inflammatory markers





Colonic oxidative stress markers



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

Propionibacteria: prevention of DSS-in

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

C57BL6
8th week age

1st Day — **5th Day** — **12th Day**

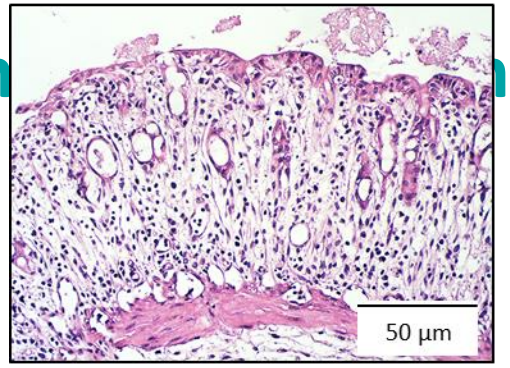
Intragastric gavage

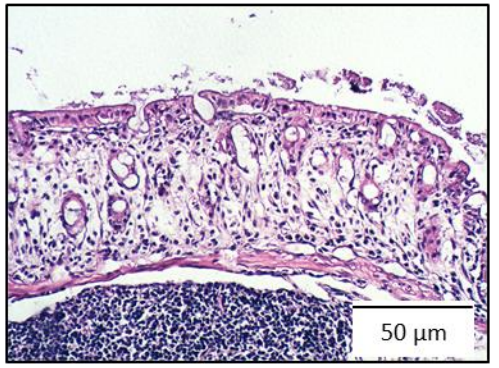
3% (w/v) **DSS solution**
(36–50 25 kDa)

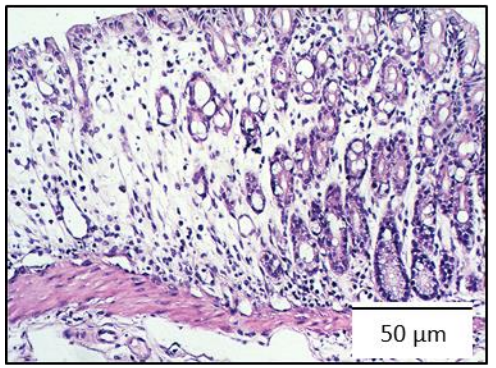
Gavage **500 µL**
(**400 mg** cheese in
500 µL PBS pH 7.4)

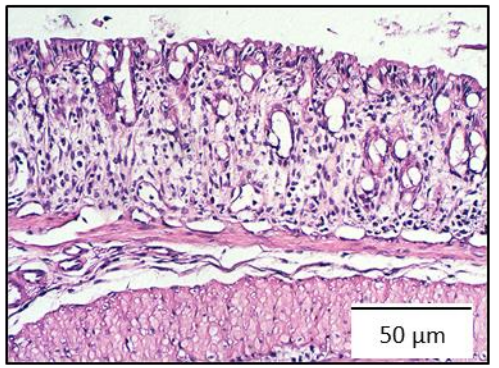
Colitis induction

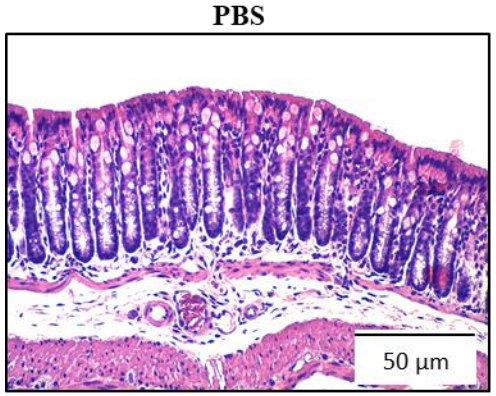
Beginning — **Euthanasia**

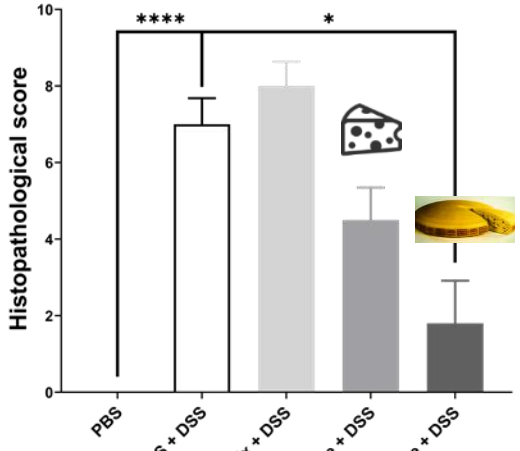








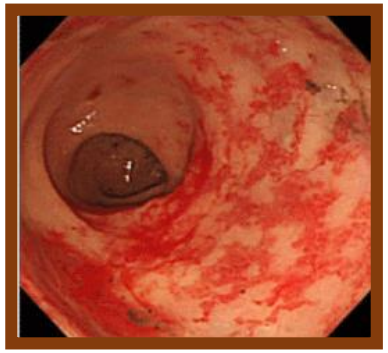




Vasco Azevedo

A growing incidence of inflammatory ailments

Mucositis



Oral mucositis

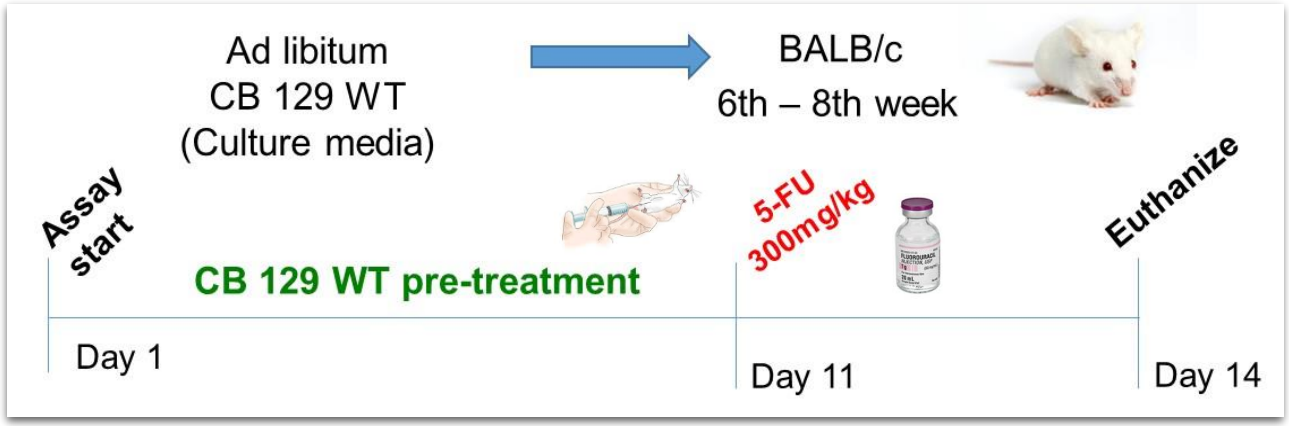
GI Mucositis

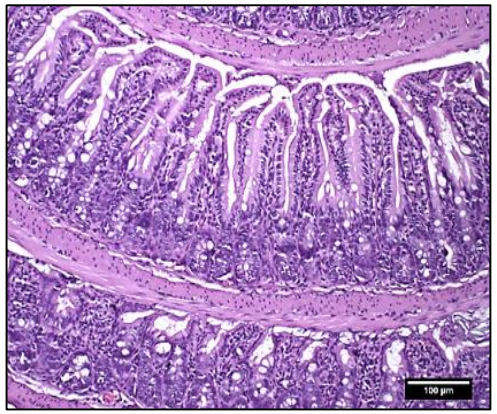
5-Fluoracil (5-FU)

- Head, neck and colon cancer.

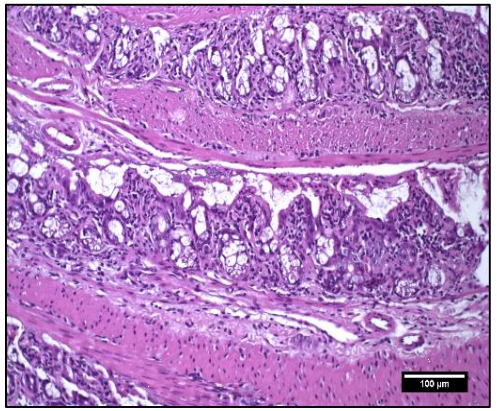
(Chang *et a*l., 2012; Falvey *et al*., 2015)

Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice

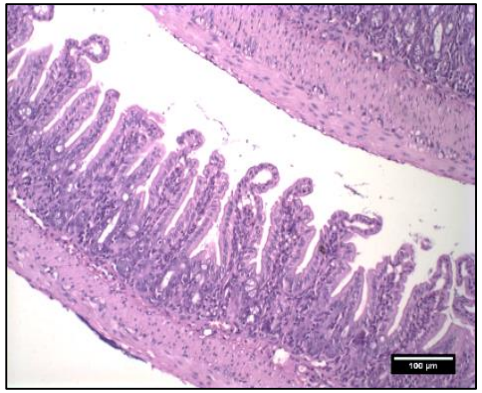




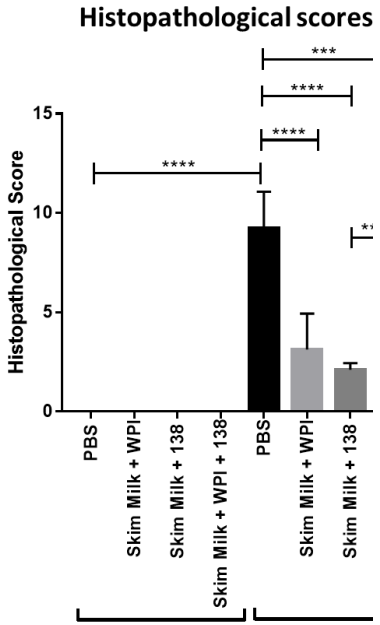
Healthy Ileum



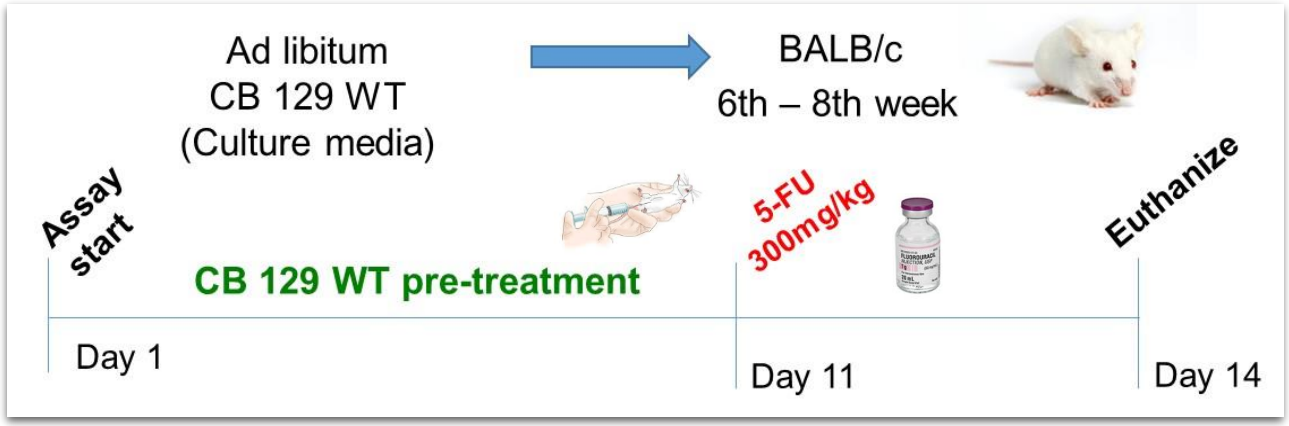
PBS + 5-FU

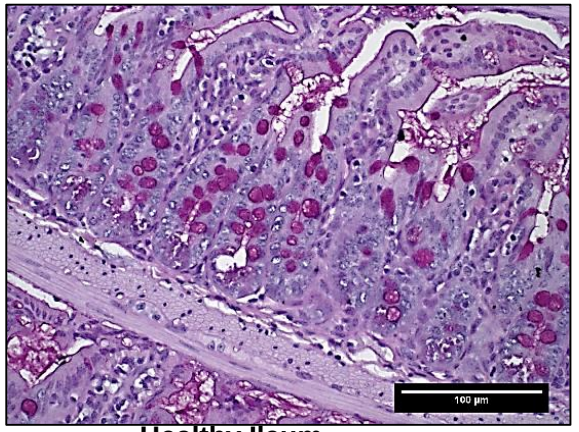


Skim milk + propio

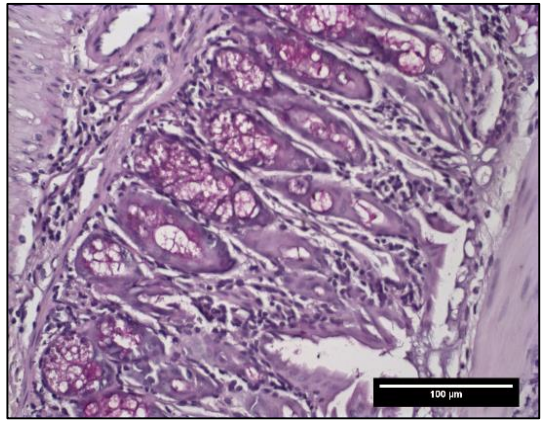


Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice

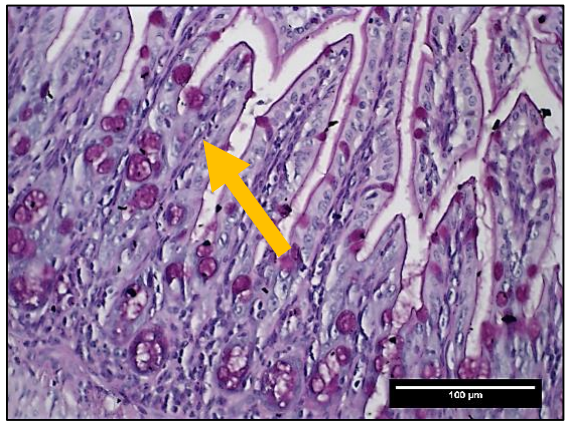




Healthy Ileum

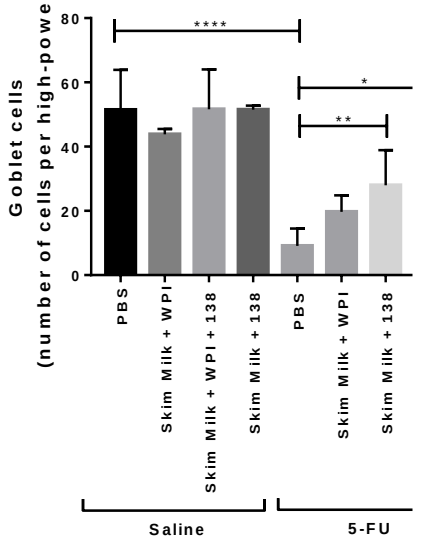


PBS + 5FU



Propio + 5FU

Goblet cells counts



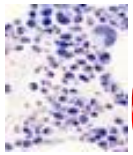
Propionibacteria modulate inflammation in pigs

30°C, 3 days

2 E+10 cfu/day

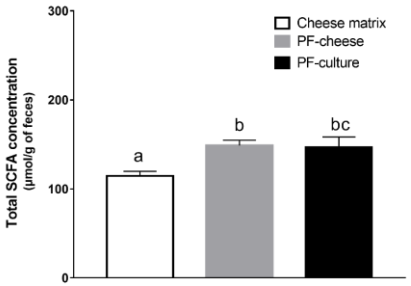
n = 16

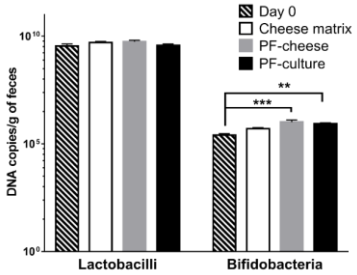
Leucocytes culture

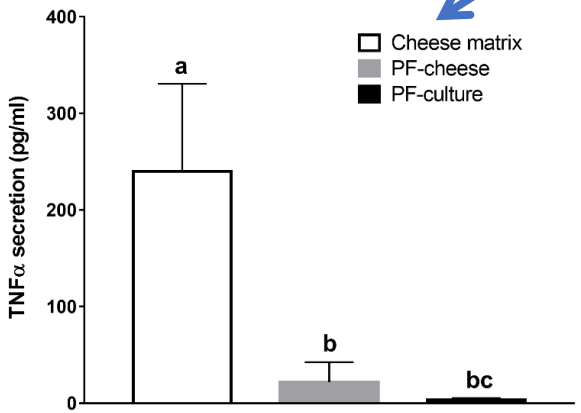


Pro-inflammatory stimulus ConA

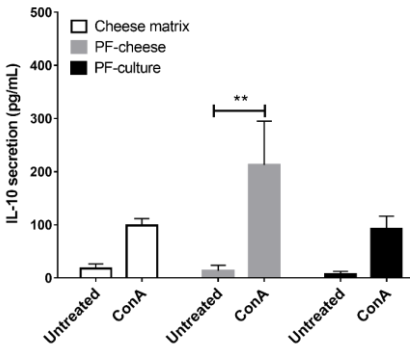
Basal secretion

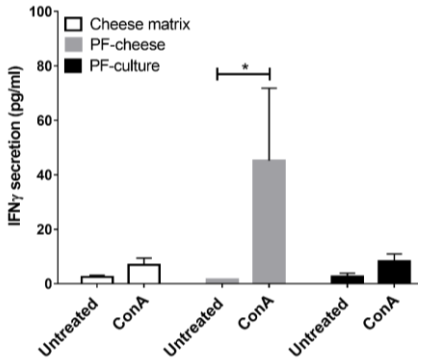






Groups	Th1/Th2 ratio	Treg/Th17 ratio
Cheese-matrix	**7180** ± 1475 [a]	**1.20** ± 0.32 [a]
PF-cheese	**2600** ± 801.3 [b]	**5,45** ± 2,08 [ab]
PF-culture	**4008** ± 721.5 [ab]	**9.65** ± 3.45 [b]





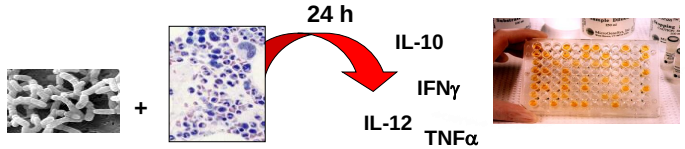
Gaëlle Boudry

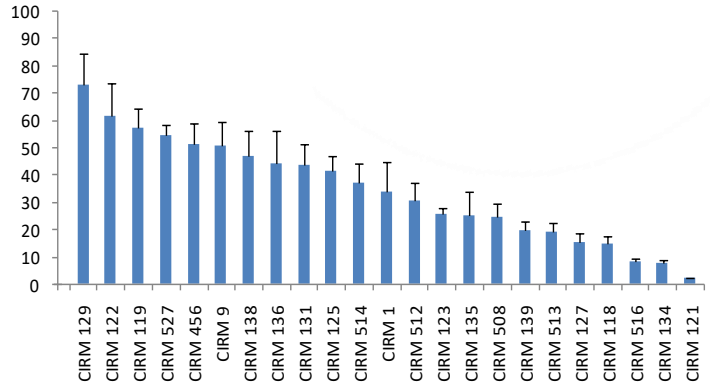
How does it work ?

Proteins involved ?

Pathways involved ?

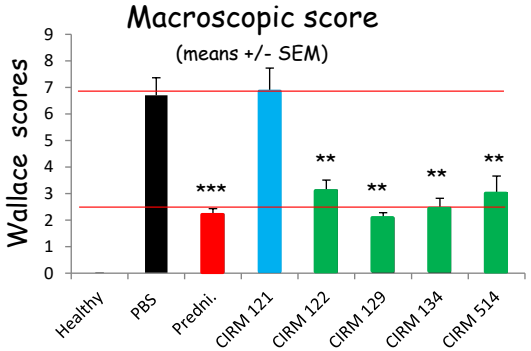
It is strain-dependent



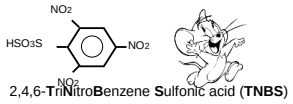


***In vitro* : IL-10**

***In vivo* : colitis**

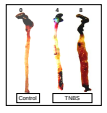


benoit.foligne@ibl.cnrs.fr

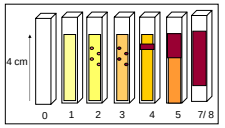


2,4,6-**T**ri**N**itro**B**enzene **S**ulfonic acid (**TNBS**)

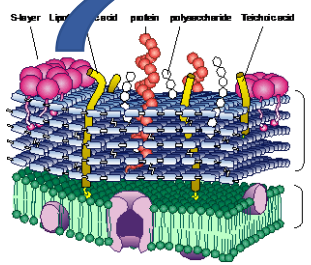
Acute colitis

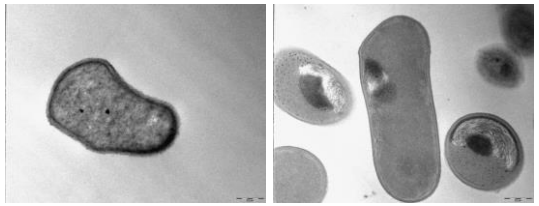


Macroscopic score (Wallace)



A key role of surface proteins





Untreated Guanidine treated

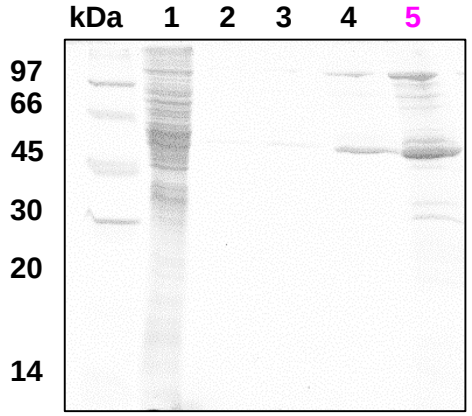
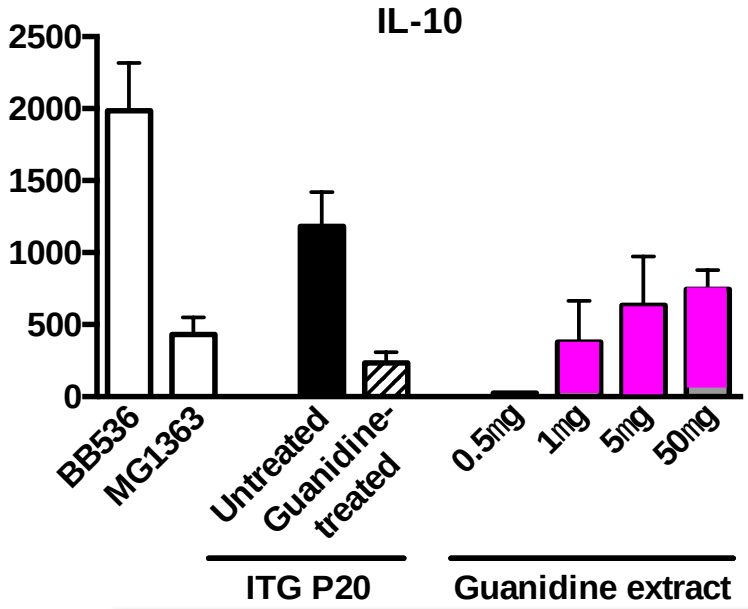


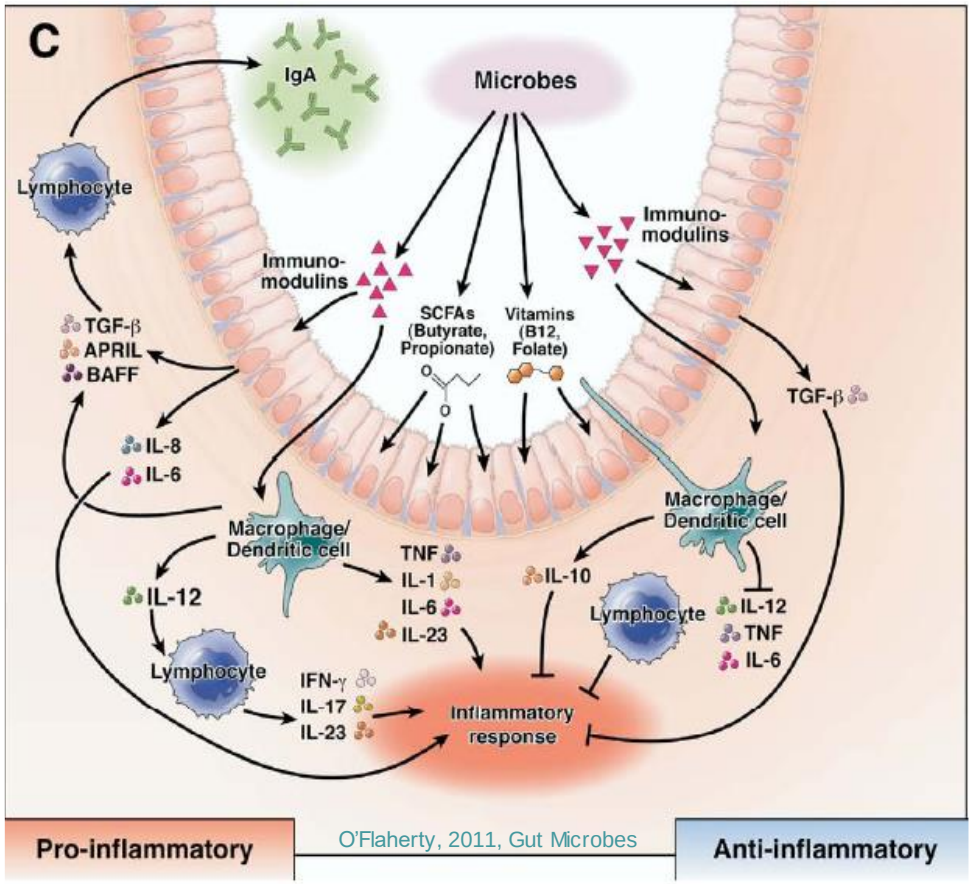
Table 1 – *Propionibacterium freudenreichii* proteins identified by nano-LC-MS/MS after guanidine hydrochl (shaving column) or *in-situ* fluorescence labeling [a] (CyDye column).

Locus Tag	Description	Gene	Function
PFCIRM129_12235	Internalin A	inlA	Miscellaneous
PFCIRM129_05460	Surface protein with SLH domain	slpE	Cell wall
FCIRM129_09350	Surface layer protein A	slpA	Cell wall
PFCIRM129_00700	Surface layer protein B	slpB	Cell wall
PFCIRM129_11445	Large surface protein A	lspA	Cell wall

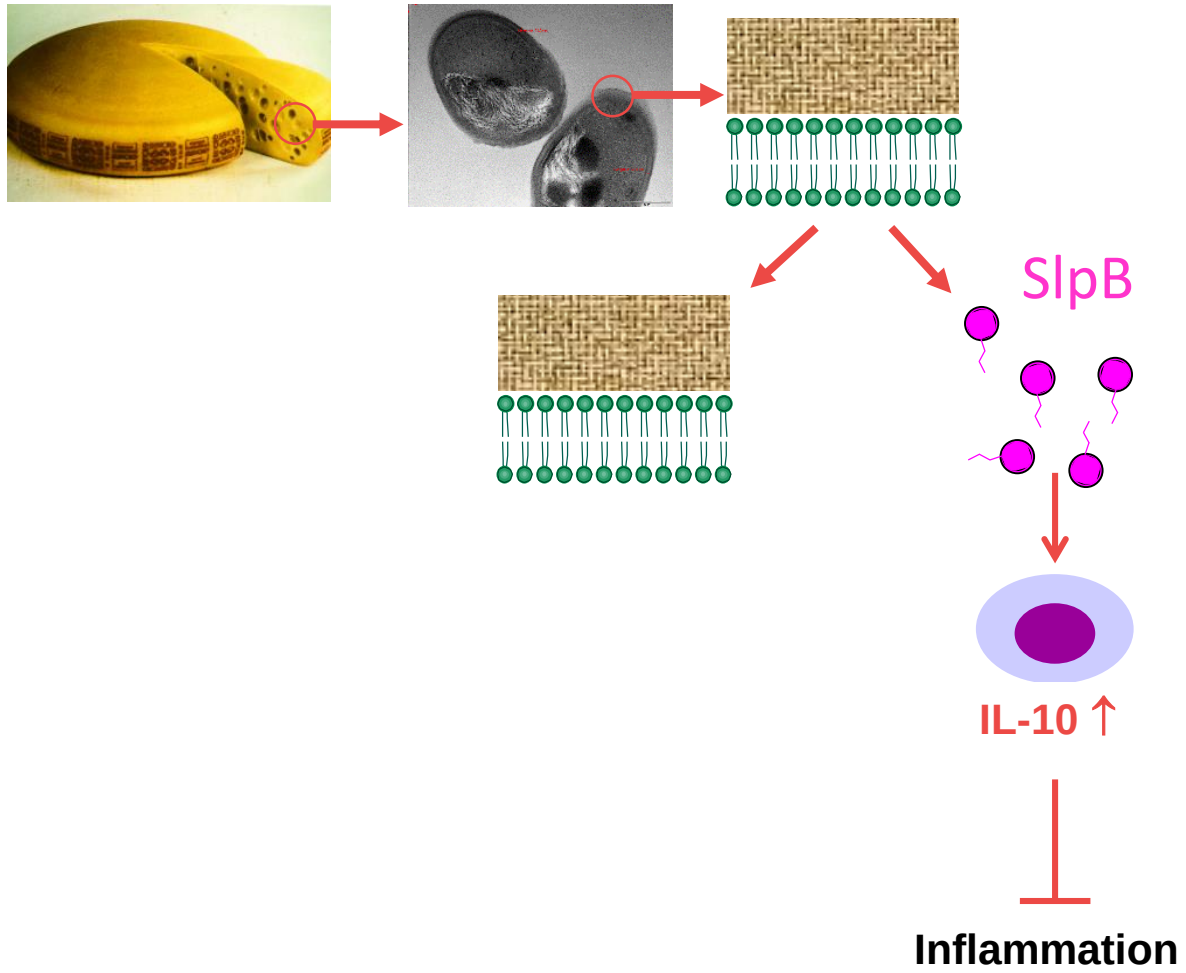


Surface proteins induce production of IL-10 in PBMC cells.

The proposed mechanism for SlpB

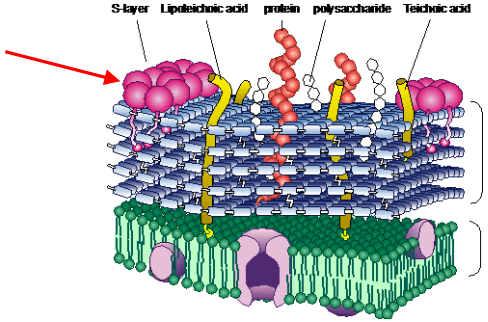


O'Flaherty, 2011, Gut Microbes

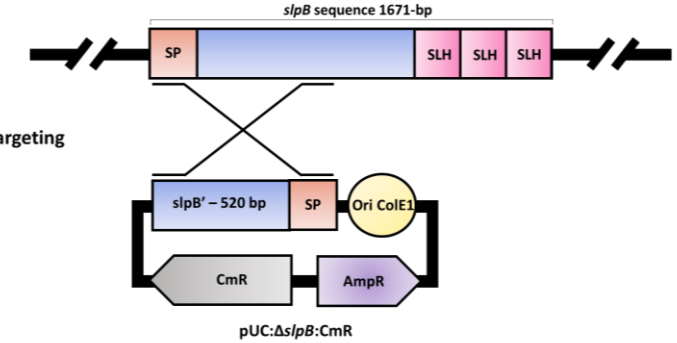


Consequences of SlpB inactivation

SlpB gene knock out



A. *slpB* gene targeting in CB 129 strain



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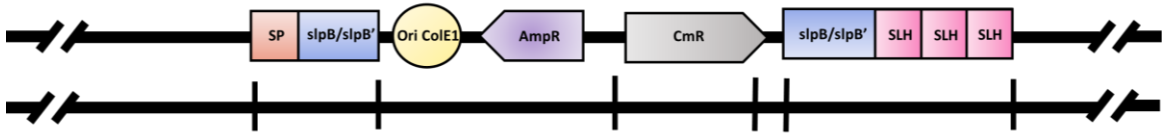
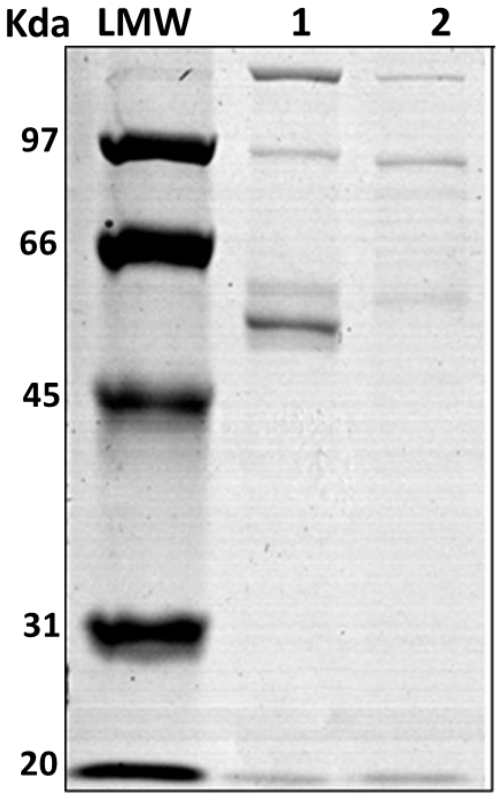
C. Targeted interruption of *slpB* gene in CB 129 strain

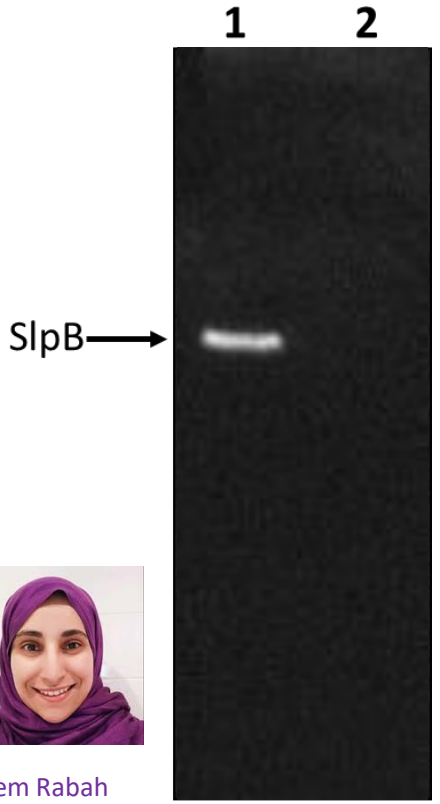


D. CB 129 *slpB* gene sequence targeting for inactivation

B

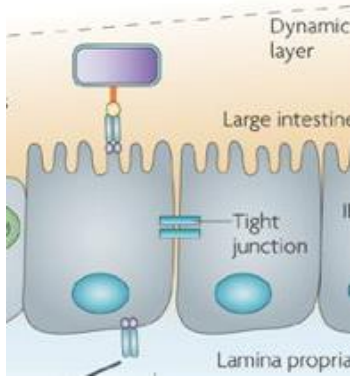


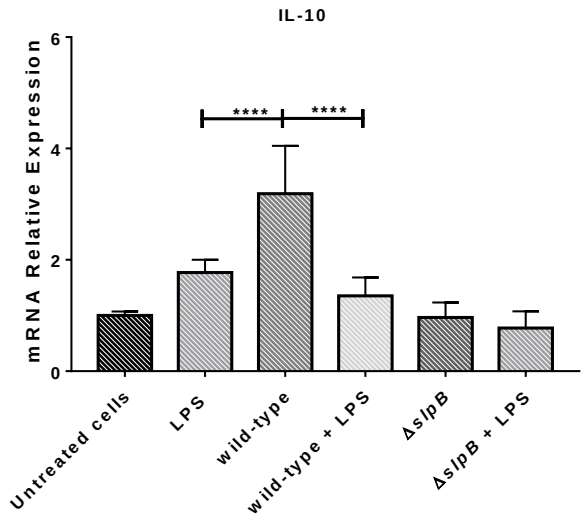
C

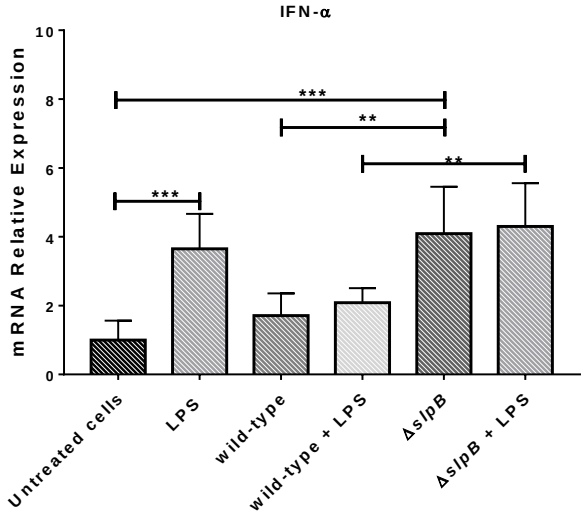


Houem Rabah
houyemrabah1990@gmail.com

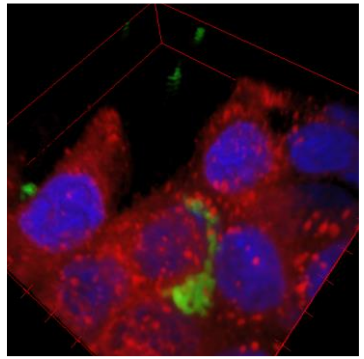
Gene knock out changes immune modulation

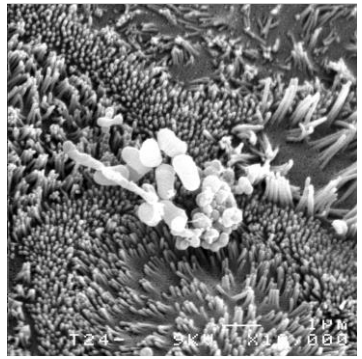


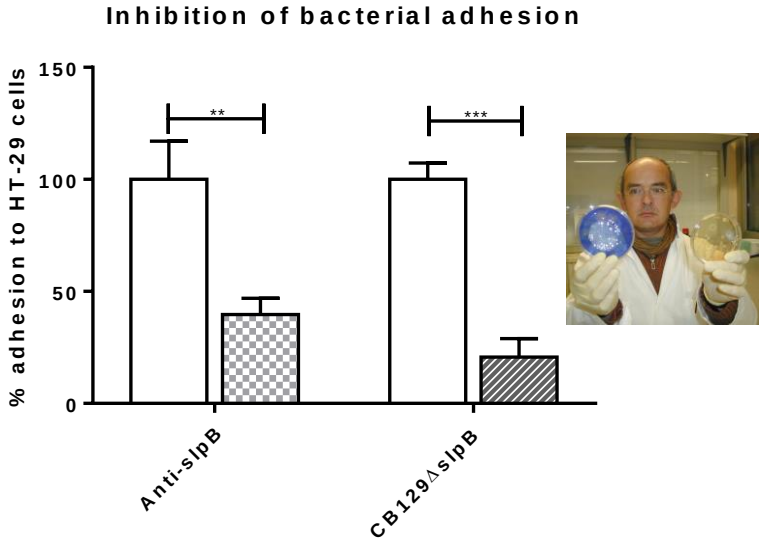




Gene knock out changes adhesion

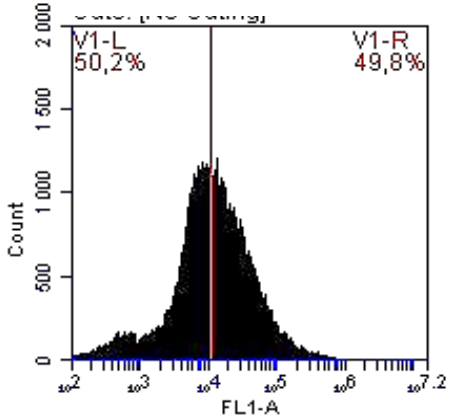




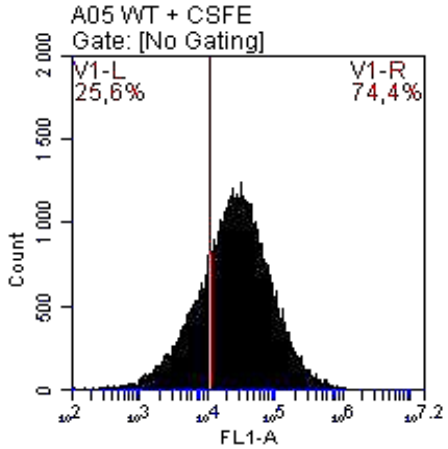


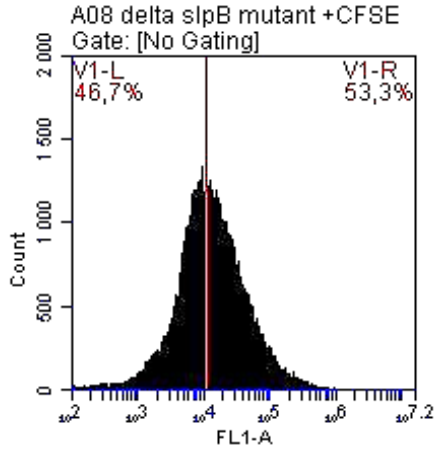
Martine.Deplanche@inra.fr

Cells alone



Cells + fluorescent WT propionis



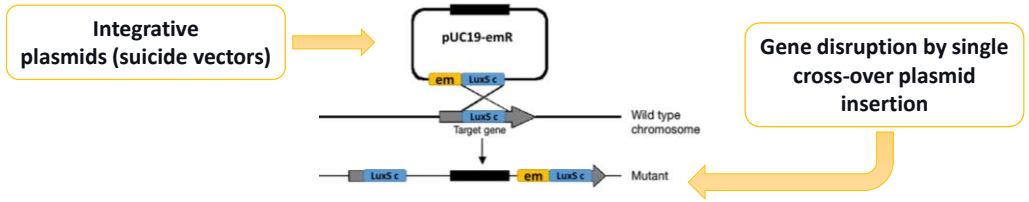


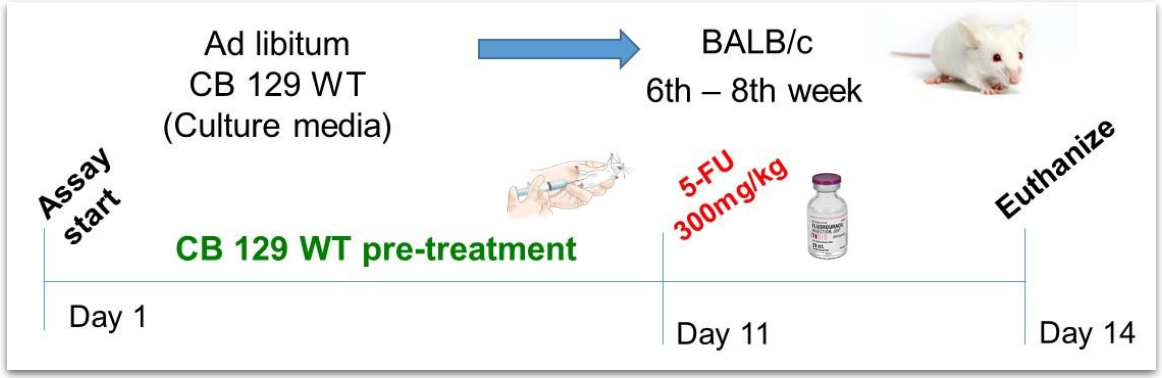
Read more : Do Carmo et al., Frontiers in Microbiology, 2017 &2018

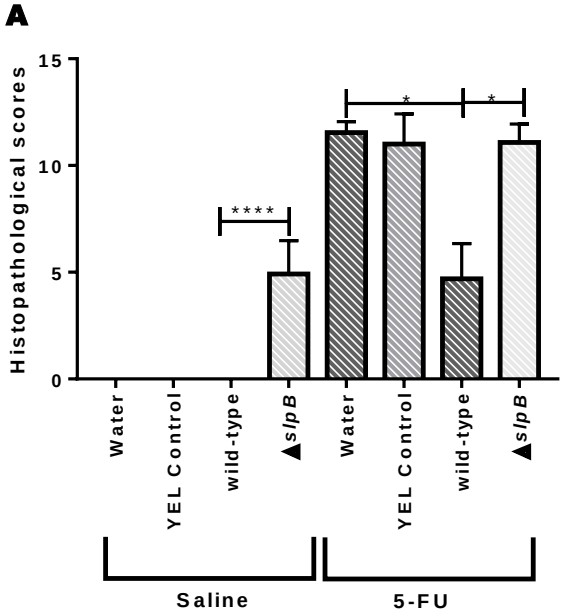
What about in vivo consequences of SlpB inactivation ?

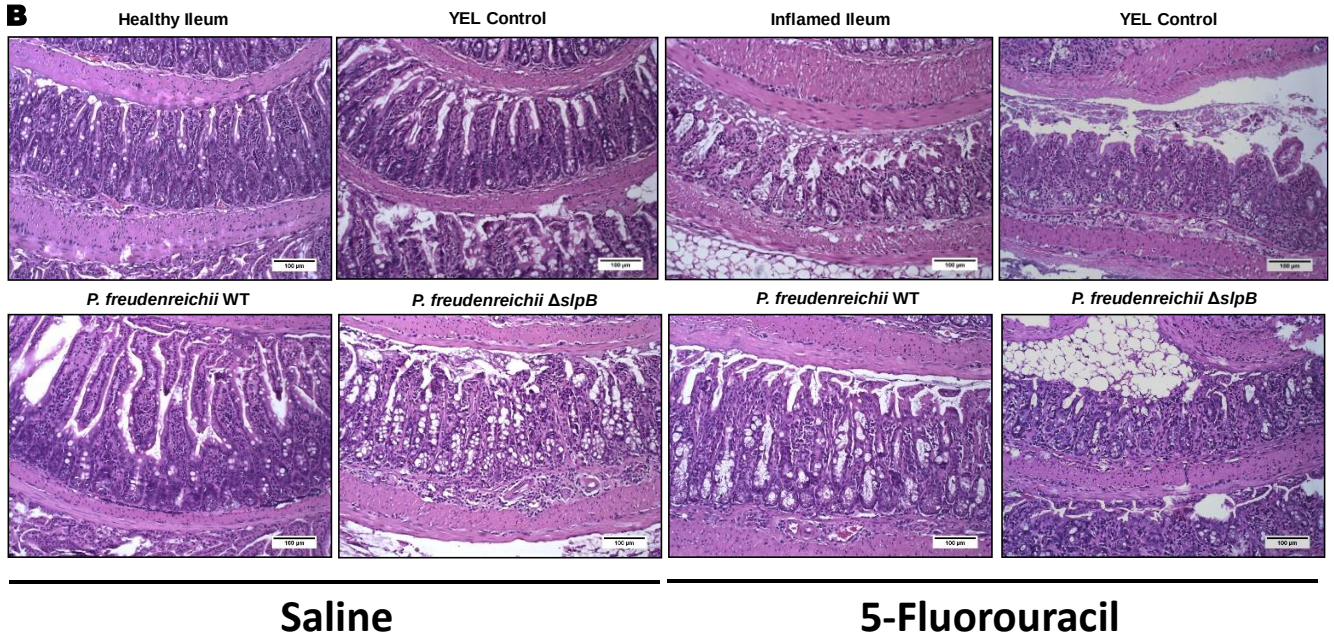


Propionibacteria: a key role of Surface Layer Protein B in probiotic effect









Mutation of SlpB suppresses protection / mucositis

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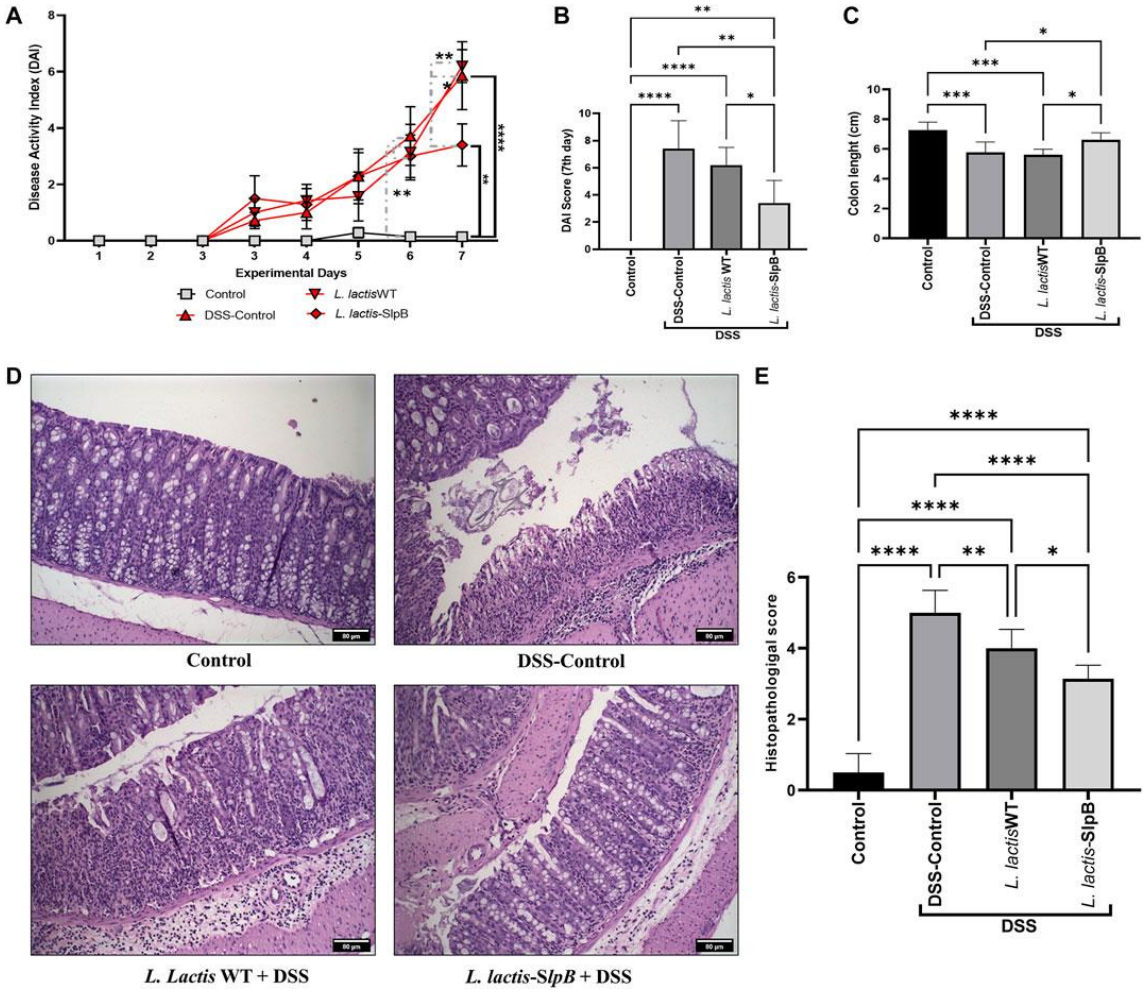
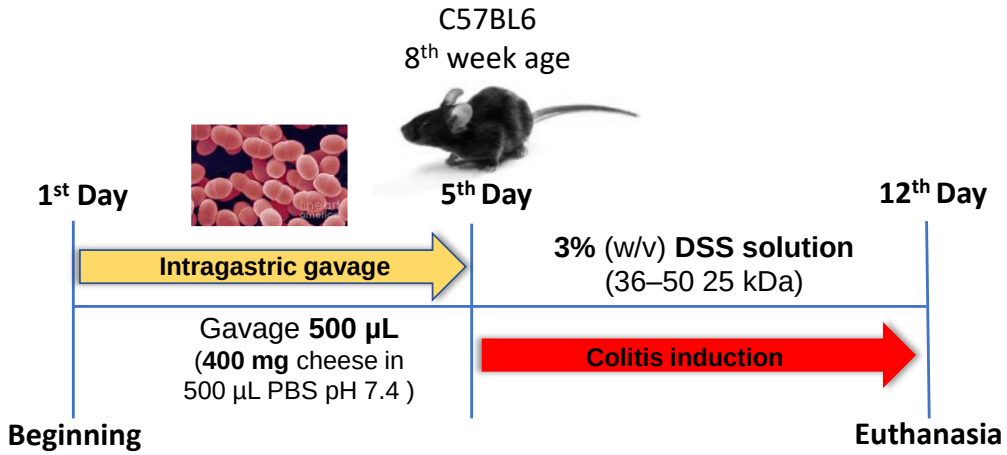
If we express SlpB in *Lactococcus lactis*: colitis challenge





SlpB expression provides *L. lactis* with enhanced anti-inflammatory properties

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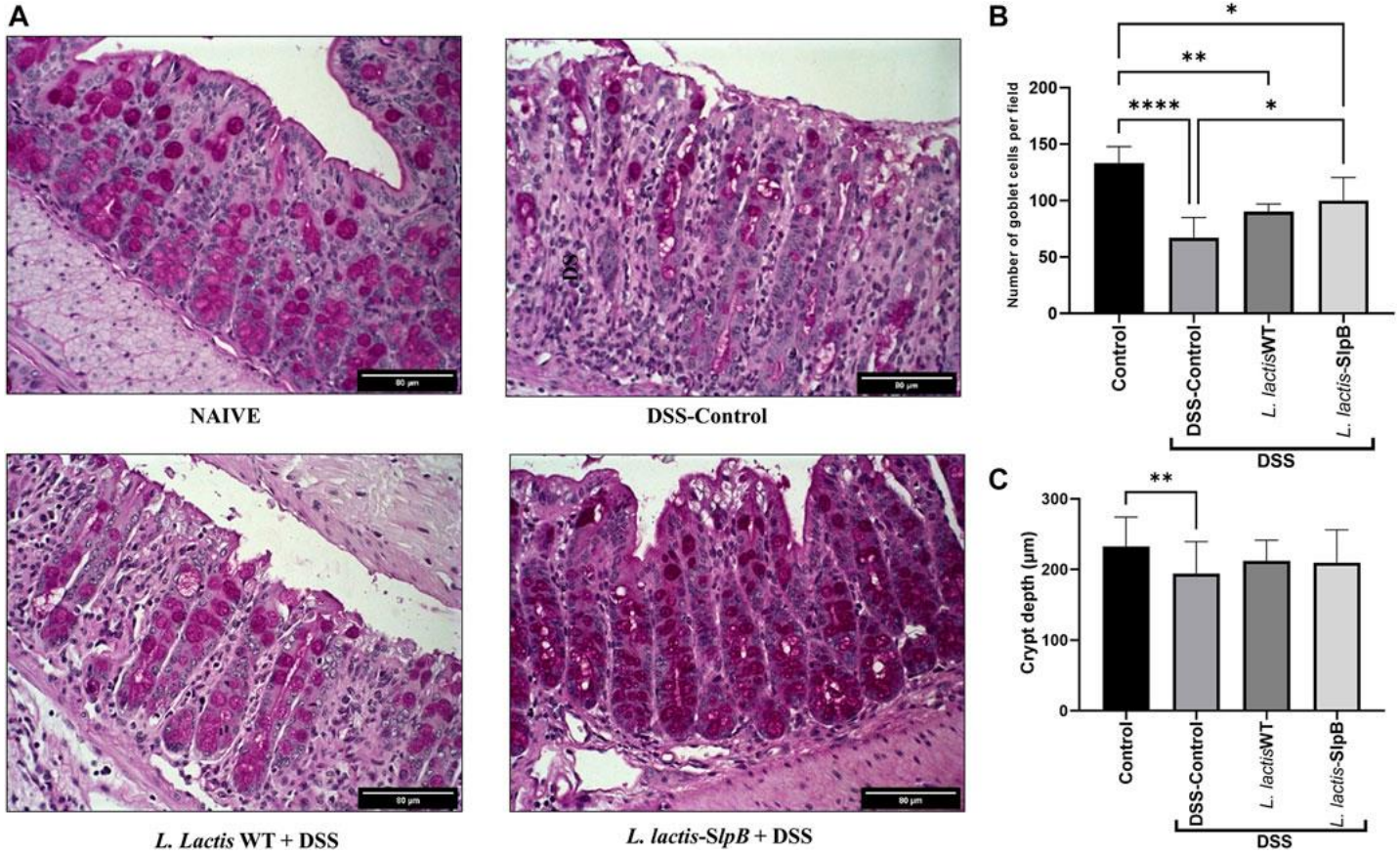
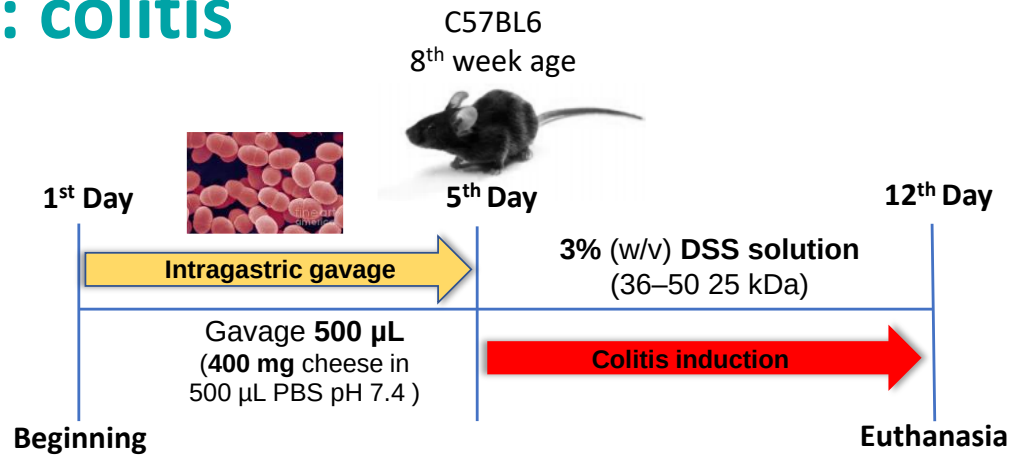
If we express SlpB in *Lactococcus lactis*: colitis





SlpB expression protects from mucus and goblet cells depletion

Do Carmo et.al., 2021, **Front. Pharmacol.** SlpB Protein Enhances the Probiotic Potential of L. lactis NCDO 2118 in Colitis Mice Model

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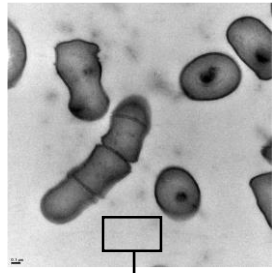
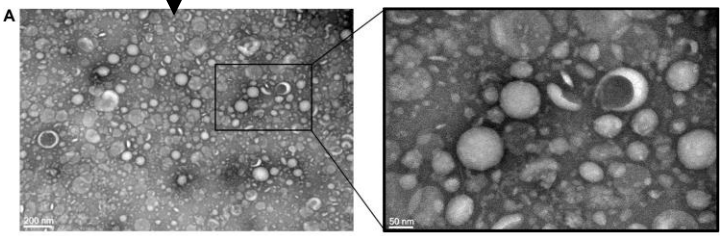
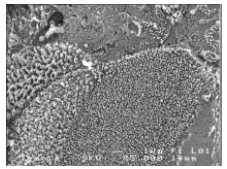
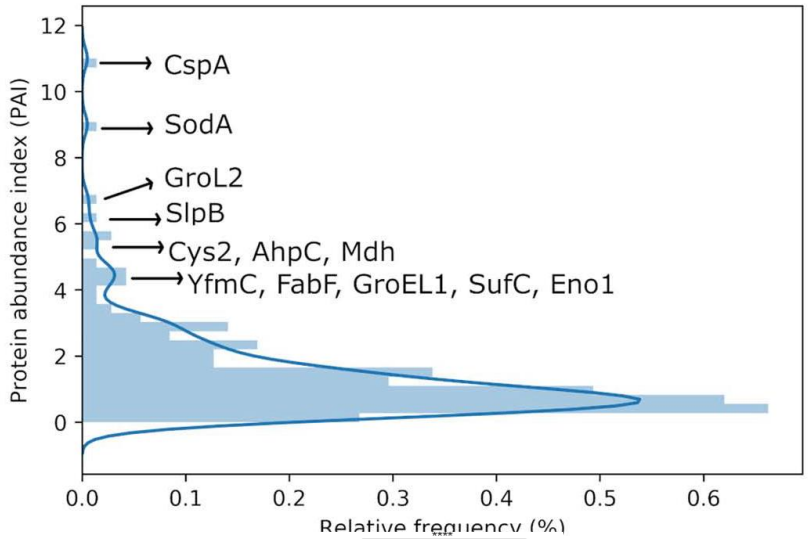
Propionibacteria: a potential role of Extracellular Vesicles







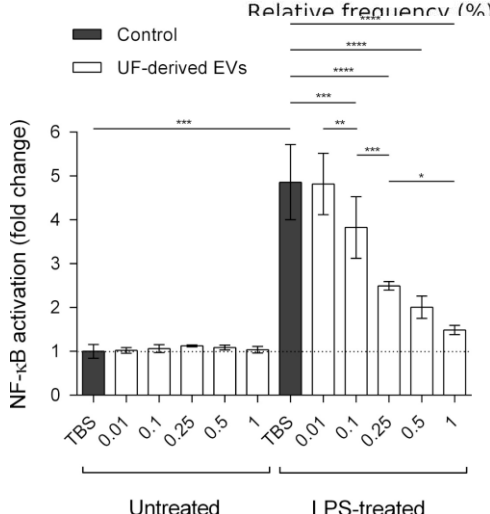
Cultured human intestinal cells

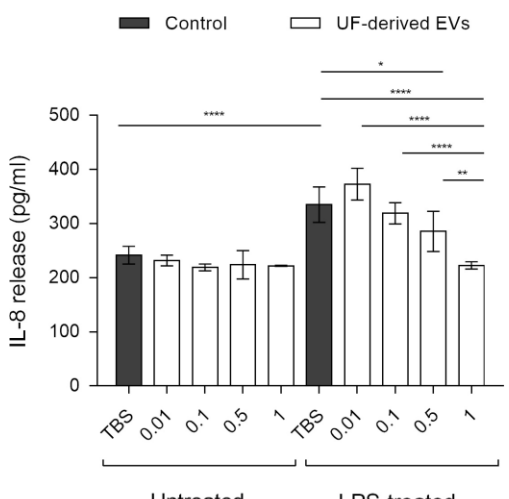


P. freudenreichii Evs contain SlpB and mitigate NF-kB as well as IL-8 in HT-29

Rodovlaho et.al., 2020, **Front. Microbiol.** Extracellular vesicles produced by the probiotic Propionibacterium freudenreichii CIRM-BIA129 mitigate inflammation by modulating the NF-κB pathway

Rodovlaho et.al., 2021, **Appl. Environ Microbiol.** Environmental conditions modulate the protein content and immunomodulatory activity of extracellular vesicles produced by the probiotic Propionibacterium freudenreichii.





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Eric Guédon, INRAE, STLO

INRAE

Probiotic diary Propionibacteria

G. Jan

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L'INSTITUT agro

Propionibacteria: a pilot clinical study on ulcerative colitis patients

ELSEVIER

NUTRITION

Nutrition 22 (2006) 76–81

www.elsevier.com/locate/nut

Preliminary report

Bifidogenic growth stimulator for the treatment of active ulcerative colitis: a pilot study

Asuka Suzuki, M.D., Ph.D.[a,b], Keiichi Mitsuyama, M.D., Ph.D.[a,b,*], Hironori Koga, M.D., Ph.D.[a,b], Nobuo Tomiyasu, M.D., Ph.D.[a,b], Junya Masuda, M.D.[a,b], Kosuke Takaki[a], Osamu Tsuruta, M.D., Ph.D.[a], Atsushi Toyonaga[a], and Michio Sata[a]

[a] *Second Department of Medicine, Kurume University School of Medicine, Fukuoka, Japan*
[b] *Research Center for Innovative Cancer Therapy and Center of the 21th Century COE Program for Medical Science, Kurume University, Fukuoka, Japan*

Manuscript received September 6, 2004; accepted April 19, 2005.

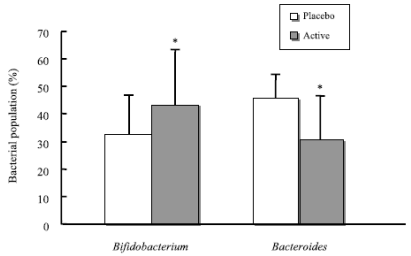


Fig. 5. Bacterial population in the feces of 41 patients who received 4.5 g of bifidogenic growth stimulator or placebo daily for 4 weeks. The *Bifidobacterium* population significantly increased and the *Bacteroides* population significantly decreased in volunteers who ingested bifidogenic growth stimulator as compared with volunteers who ingested placebo.
Values represent the mean ± SD.
*: Significantly different from the placebo group ($p < 0.05$) (*Ref. 14*).

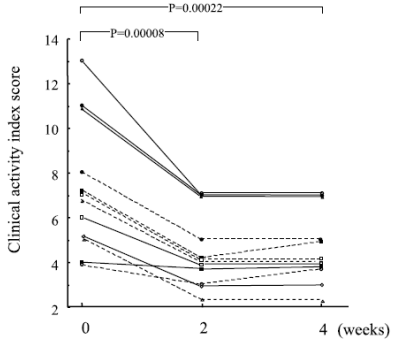
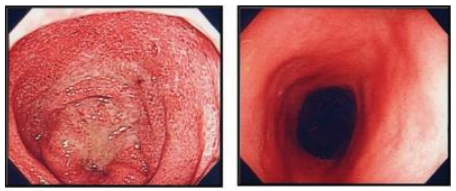


Fig. 6. Serial changes in clinical activity index scores in 12 patients who had ulcerative colitis during the observation period. Patients with active ulcerative colitis received 4.5 g of bifidogenic growth stimulator daily for 4 weeks. Clinical activity index scores were significantly improved by the ingestion of bifidogenic growth stimulator. (*Ref. 15*)



But… n=12, no placebo….

Propionibacteria commensals of the human gut microbiota: next generation probiotics?

Consumption of human breast milk attenuates the incidence of NEC

RESEARCH ARTICLE

The Journal of Clinical Investigation

Commensal *Propionibacterium* strain UF1 mitigates intestinal inflammation via Th17 cell regulation

Natacha Colliou,[1,2] Yong Ge,[1,2] Bikash Sahay,[1] Minghao Gong,[1,2] Mojgan Zadeh,[1,2] Jennifer L. Owen,[3] Josef Neu,[4] William G. Farmerie,[5] Francis Alonzo III,[6] Ken Liu,[7] Dean P. Jones,[7] Shuzhao Li,[7] and Mansour Mohamadzadeh[1,2]

[1]Department of Infectious Diseases and Immunology, [2]Division of Gastroenterology, Hepatology & Nutrition, Department of Medicine, [3]Department of Physiological Sciences, [4]Division of Neonatology, Department of Pediatrics, and [5]Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida, USA. [6]Department of Microbiology and Immunology, Loyola University Chicago, Maywood, Illinois, USA. [7]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA.

Consumption of human breast milk (HBM) attenuates the incidence of necrotizing enterocolitis (NEC), which remains a leading and intractable cause of mortality in preterm infants. Here, we report that this diminution correlates with alterations in the gut microbiota, particularly enrichment of *Propionibacterium* species. Transfaunation of microbiota from HBM-fed preterm infants or a newly identified and cultured *Propionibacterium* strain, P. UF1, to germfree mice conferred protection against pathogen infection and correlated with profound increases in intestinal Th17 cells. The induction of Th17 cells was dependent on bacterial dihydrolipoamide acetyltransferase (DlaT), a major protein expressed on the P. UF1 surface layer (S-layer). Binding of P. UF1 to its cognate receptor, SIGNR1, on dendritic cells resulted in the regulation of intestinal phagocytes. Importantly, transfer of P. UF1 profoundly mitigated induced NEC-like injury in neonatal mice. Together, these results mechanistically elucidate the protective effects of HBM and P. UF1–induced immunoregulation, which safeguard against proinflammatory diseases, including NEC.

This correlates with alteration in the gut microbiota

Particularly enrichment of *Propionibacterium* species

A *Propionibacterium* strain isolated from healthy breast-fed new-born protects mice against pathogen infection and against induced colitis

It triggers a profound increase in intestinal Th17 cells

It binds to SIGNR1 receptor on dendritic cells

Surface layer proteins are involved

- Propionibacteria: strain-dependent immunomodulatory properties
- Ability to mitigate the pro-inflammatory response
- Surface layer proteins play a key role in this interaction
- This can be transferred to another bacterium via heterologous expression
- Propionibacteria, members of the human gut microbiota symbionts (?)
- Commensal strains of propionibacteria, a perspective for IBD treatment ?







INRAE




Thank you for your attention!
Any kind question?

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