



HAL
open science

Fat matters: fermented whole milk potentiates the anti-colitis effect of *Propionibacterium freudenreichii*

Marine Mantel, Tales Fernando Da Silva, Rafael Gloria, Danièle Vassaux, Kátia Duarte Vital, Valbert Nascimento Cardoso, Eric Guédon, Simone Odília Antunes Fernandes, Yves Le Loir, Ana Maria Caetano Faria, et al.

► To cite this version:

Marine Mantel, Tales Fernando Da Silva, Rafael Gloria, Danièle Vassaux, Kátia Duarte Vital, et al.. Fat matters: fermented whole milk potentiates the anti-colitis effect of *Propionibacterium freudenreichii*. Galactinov: Rendez-vous annuel Op+lait 2023, Réseau de recherche à l'international (2RI) GALACTINNOV, Oct 2023, Québec (online), France. hal-04249944

HAL Id: hal-04249944

<https://hal.inrae.fr/hal-04249944>

Submitted on 19 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

➤ Le gras, ça compte : le lait entier favorise l'effet anti-inflammatoire de *Propionibacterium freudenreichii*

Gwénaél JAN

STLO, INRAE, Institut Agro, Science et Technologie du Lait et de l'Œuf, Rennes

gwenael.jan@inrae.fr

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



➤ Emmental cheese : cooked and pressed cheese

Streptococcus thermophilus
&
Lactobacillus helveticus

Propionibacterium freudenreichii



Raw milk (1000 L)

Thermisation (90°C, 5 min)

Cooling down to 37°C

Addition of rennet

Addition of Lactic Acid Bacteria

Addition of Propionic Acid Bacteria

Clotting (coagulation)

Cutting of the curd

Cooking 55°C, 1h

Moulding, pressing, cooling down takes 24h

Salting

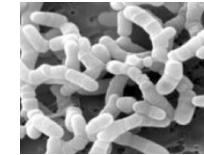
Cold room

Warm room

Ripening (weeks....to years!)



- 250,000 tons Emmental/year in France
- $10^{10} - 10^{11}$ *P. freudenreichii*/day/person
- Also present in probiotic food supplements



Valio, Finland
L. rhamnosus Lc705,
Propionibacterium freudenreichii
ssp. shermanii JS,
Bifidobacterium animalis subsp.
lactis Bb-12®.



Standa, France
Propionibacterium freudenreichii
ssp. shermanii
 Propiofidus



meiji

Meiji, Japan
Propionibacterium freudenreichii
ssp. shermanii ET-3

➤ We eat more starter bacteria than probiotics!

Letters to Nature 2014, L.A. Davis et al. : Diet rapidly and reproducibly alters the human gut microbiome!!

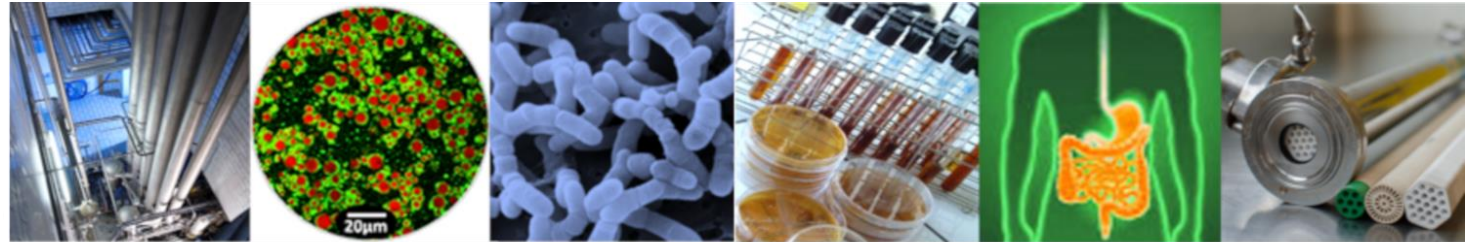


INRAE

Propionibacteria immunomodulation
 Fat matters



➤ *Propionibacterium freudenreichii*: Who's that bug?

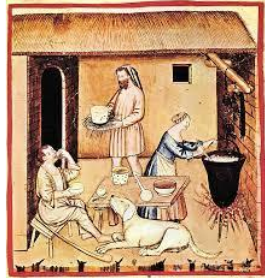


INRAE

Propionibacteria immunomodulation
Fat matters



➤ Emmental and other cheeses with holes: a long story



Flavour:

Fruity

Sour

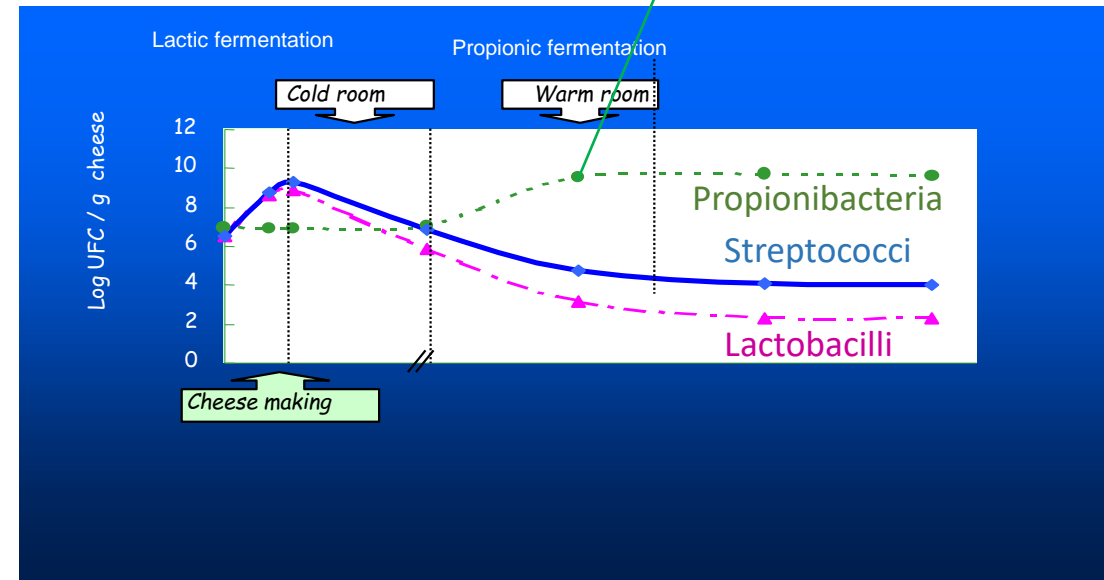
Picante

Propionate

Acetate

CO₂

...Esters



➔ The Fitz equation (1878)



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

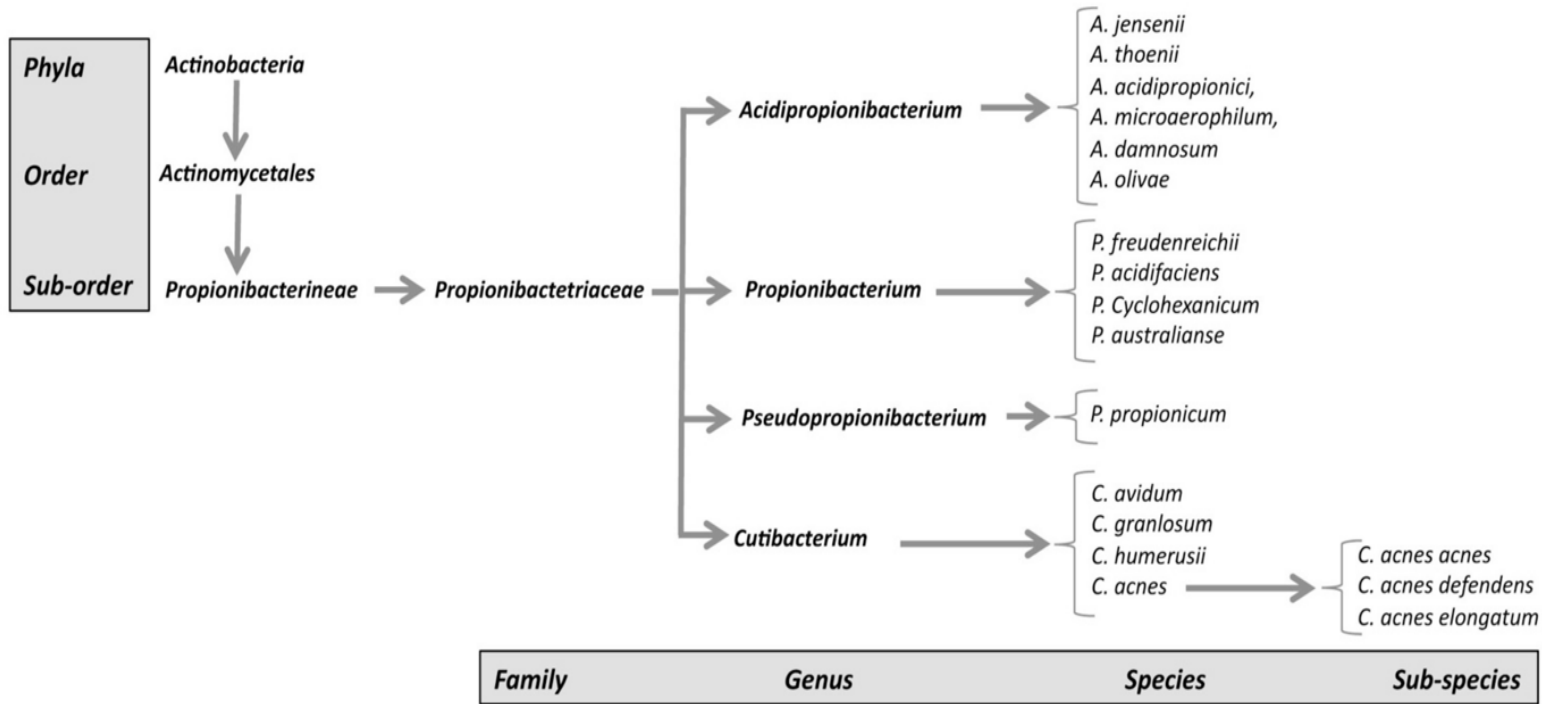


INRAE

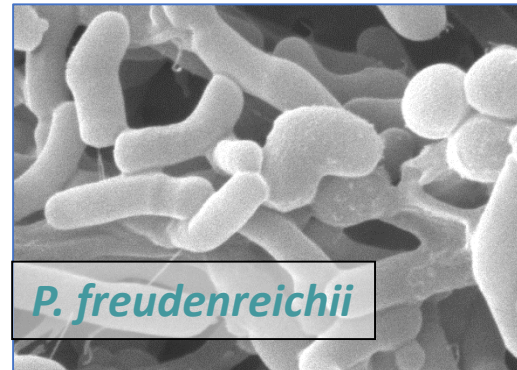
Propionibacteria immunomodulation
Fat matters



➤ Propionibacteria, in a few words



Gram +
 pleiomorphic
 anaerobic to microaérophilic
 non motiles et non sporulated

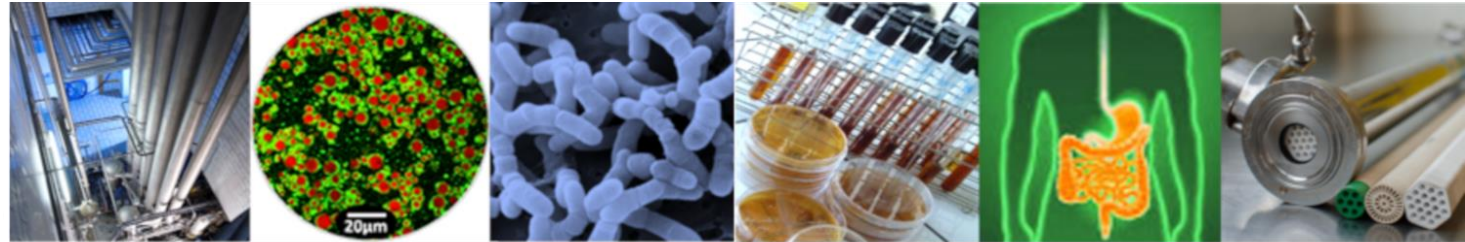


INRAE

Propionibacteria immunomodulation
 Fat matters



➤ *Propionibacterium freudenreichii*: An inducer of anti-inflammatory cytokines



INRAE

Propionibacteria immunomodulation
Fat matters



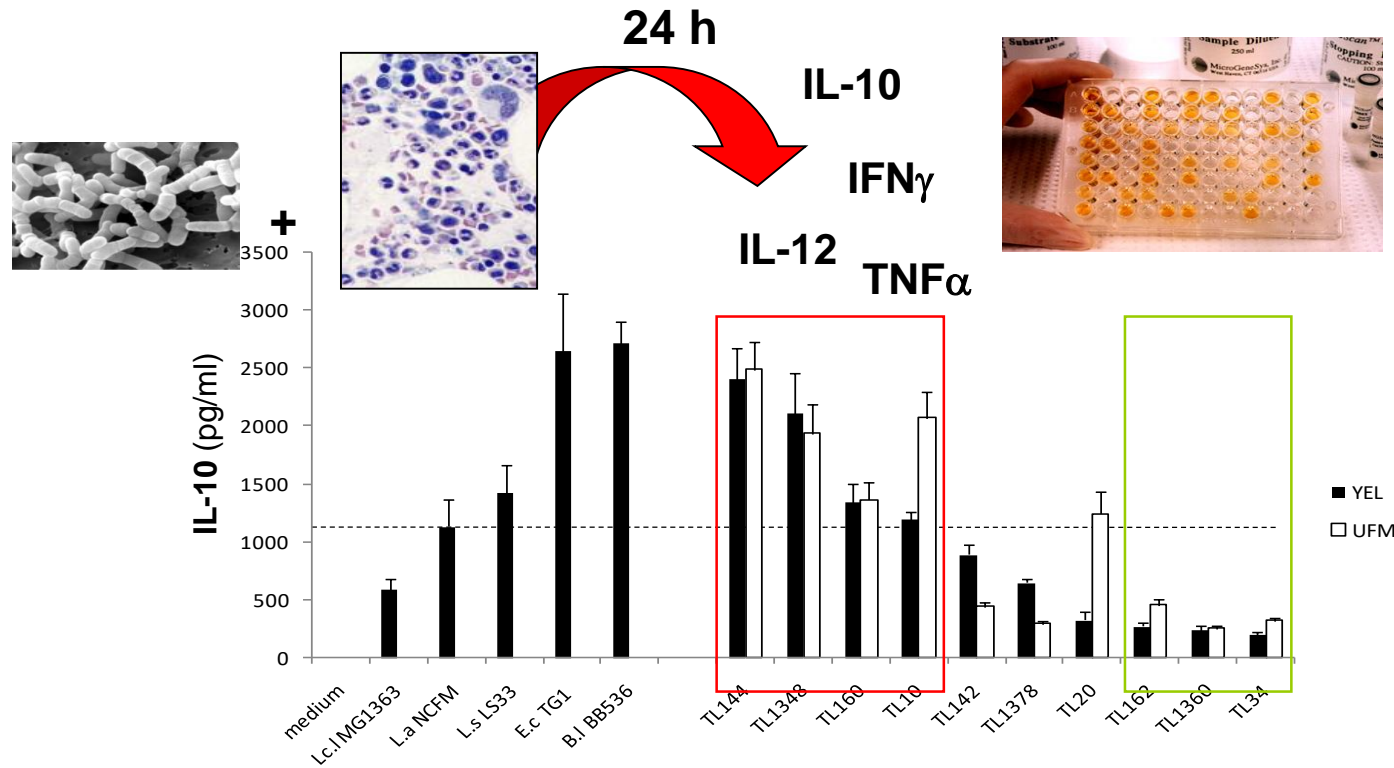
➤ Cytokine stimulation on human PBMCs (mononucleated leucocytes from donors)



Bruno Pot



Benoit Foligné



The best anti-inflammatory strains

The less anti-inflammatory strains

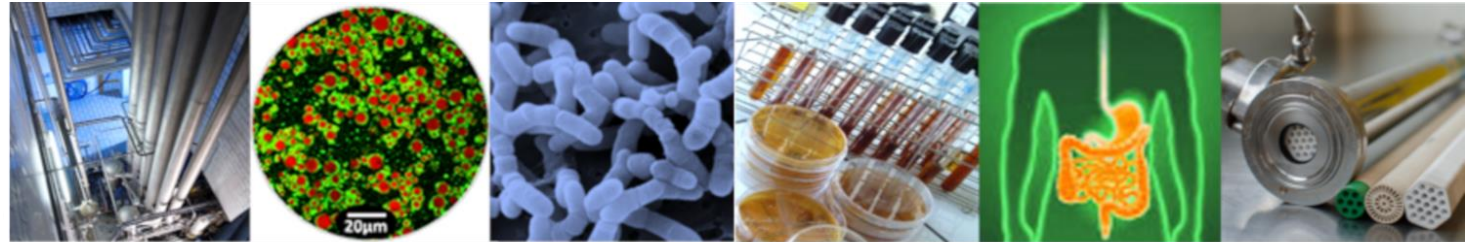


INRAE

Propionibacteria immunomodulation
Fat matters



➤ *Propionibacterium freudenreichii*: A way to fight inflammatory bowel diseases?

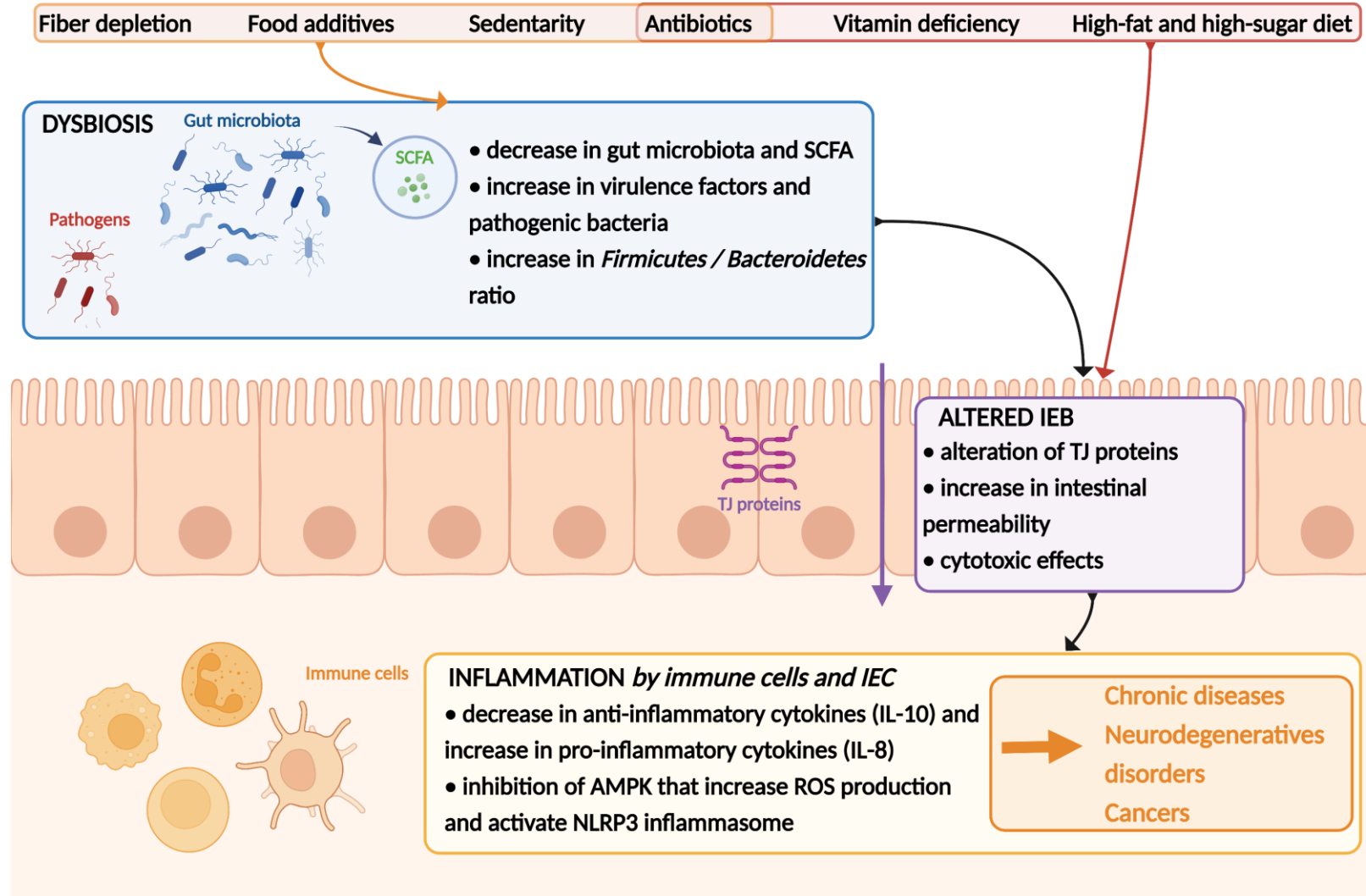


INRAE

Propionibacteria immunomodulation
Fat matters



> Inflammation of the gut : a growing concern



➤ Examples of digestive inflammatory diseases

Colitis

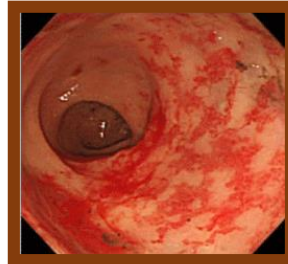
- GIT Chronic inflammation



Ulcerative Colitis



Mucositis



GI Mucositis



Oral mucositis

5-Fluoracil (5-FU)

- Head, neck and colon cancer.

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



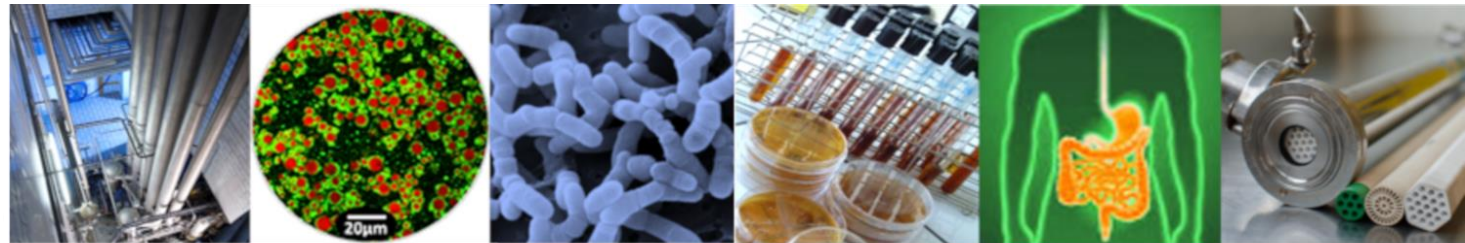
➤ *Propionibacterium freudenreichii*-fermented milk: anti-colitis effects demonstrated *in vivo*

Colitis

- GIT Chronic inflammation



Ulcerative Colitis

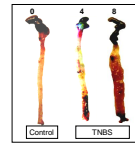
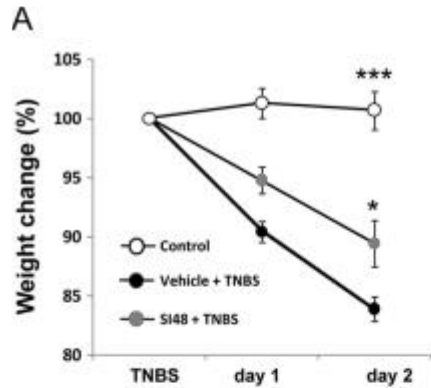


INRAE

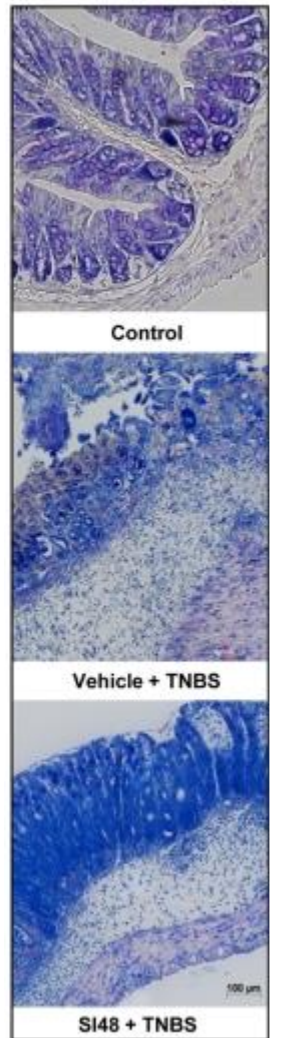
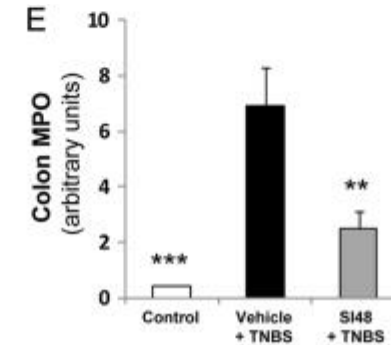
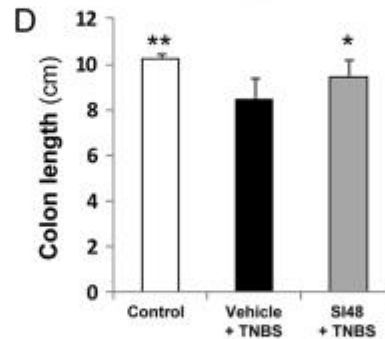
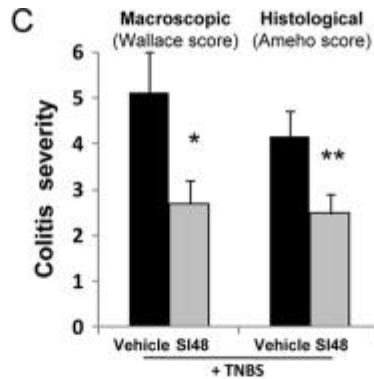
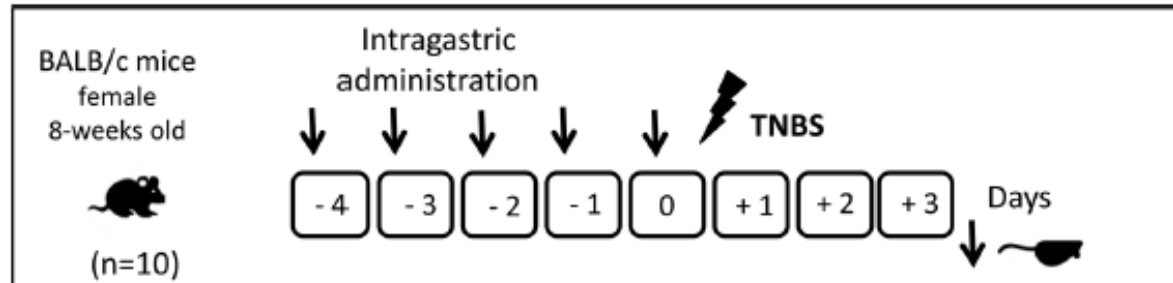
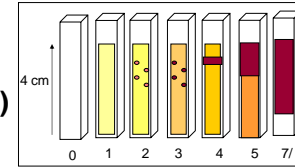
Propionibacteria immunomodulation
Fat matters



➤ Propionibacteria: prevention of TNBS-induced colitis in mice



Macroscopic score (Wallace)



Benoit Foligné



Bruno Pot



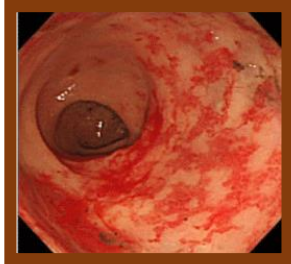
INRAE

Propionibacteria immunomodulation
Fat matters



➤ *Propionibacterium freudenreichii*-fermented milk: anti-mucositis effects demonstrated *in vivo*

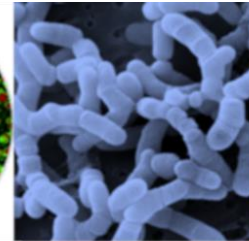
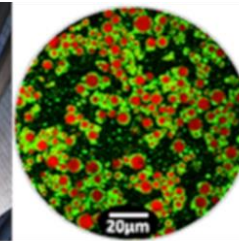
Mucositis



GI Mucositis



Oral mucositis



5-Fluoracil (5-FU)

- Head, neck and colon cancer.

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



INRAE

Propionibacteria immunomodulation

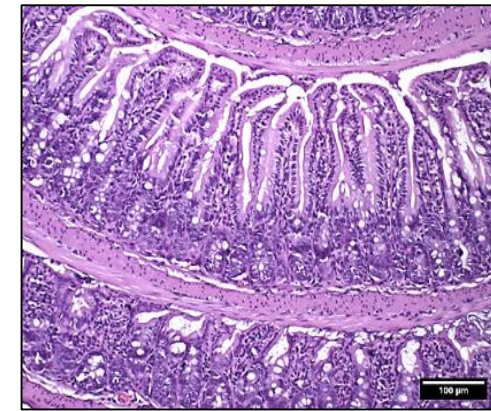
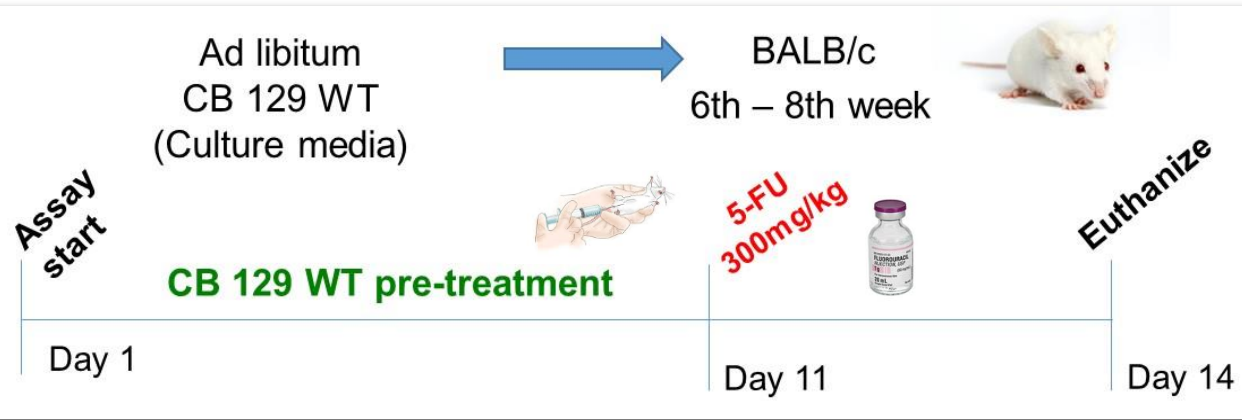
Fat matters

STLO

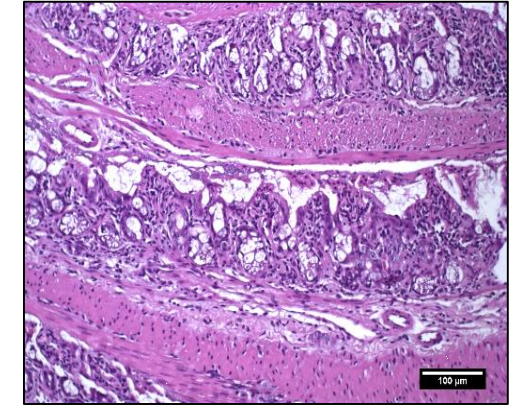
Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

L'INSTITUT
agro

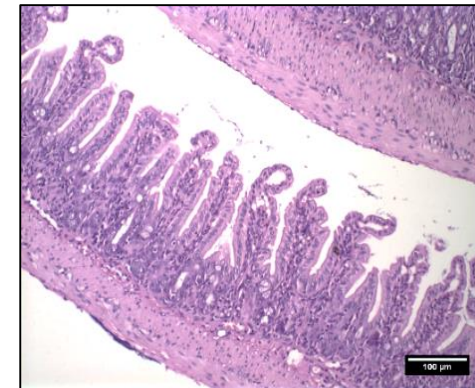
➤ Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice



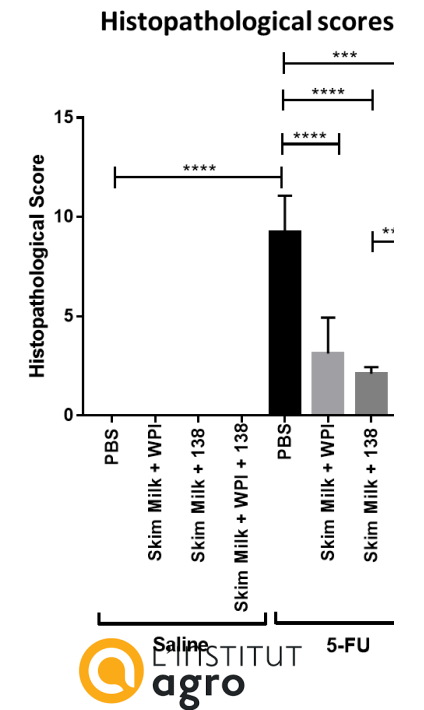
Healthy Ileum



PBS + 5-FU



Skim milk + propio



Vasco Azevedo



INRAE

Propionibacteria immunomodulation
Fat matters

UFMG
UNIVERSIDADE FEDERAL DE MINAS GERAIS

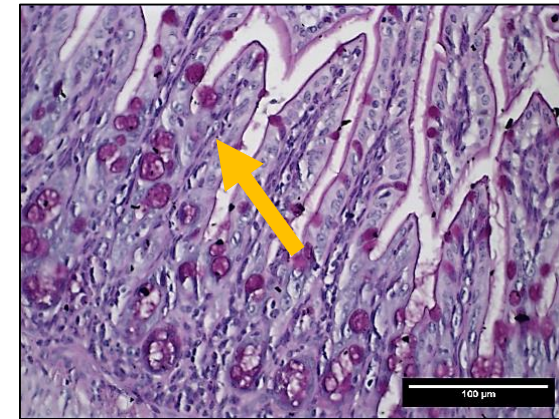
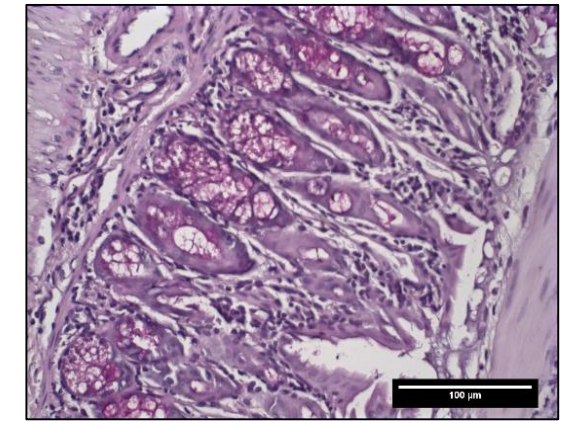
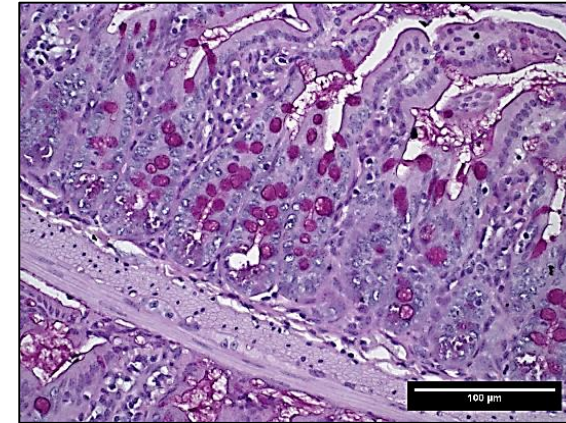
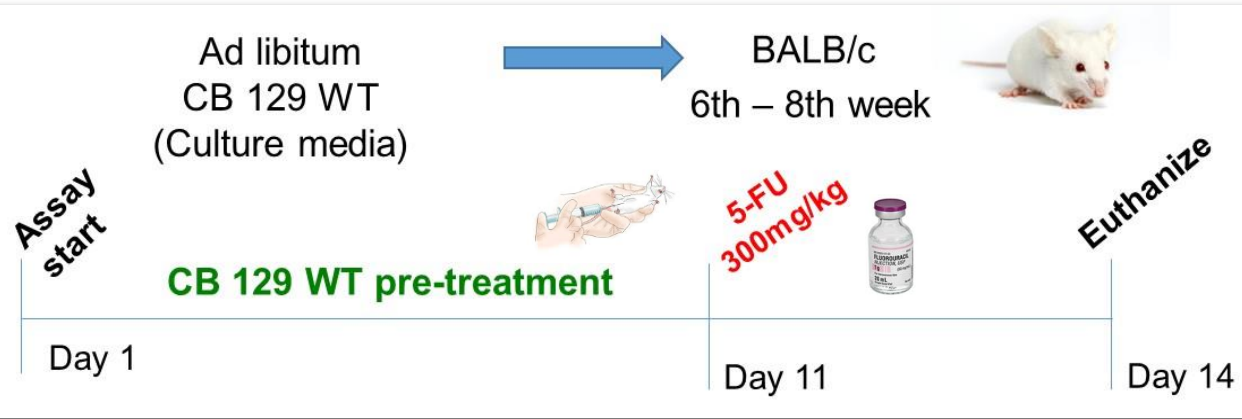
LGCM
Laboratório de Genética Celular e Molecular

STLO

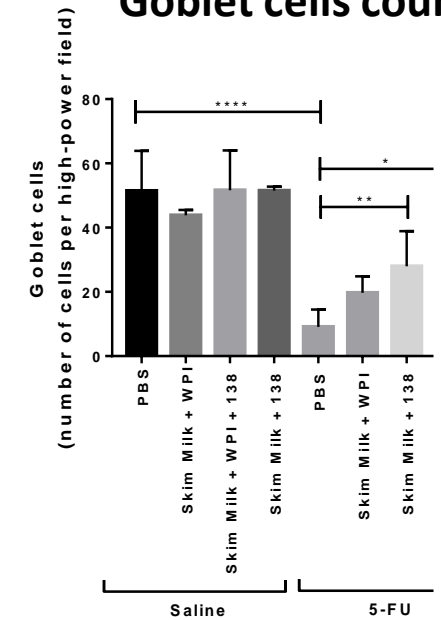


Saline Institut agro

➤ Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice



Goblet cells counts



Vasco Azevedo



INRAE

Propionibacteria immunomodulation
Fat matters

UFMG
UNIVERSIDADE FEDERAL
DE MINAS GERAIS

LGCM
Laboratório de Genética
Celular e Molecular

STLO



Liberté • Egalité • Fraternité
RÉPUBLIQUE FRANÇAISE

L'INSTITUT
agro

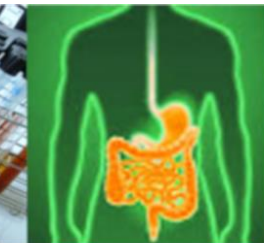
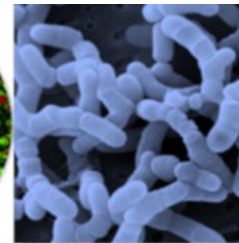
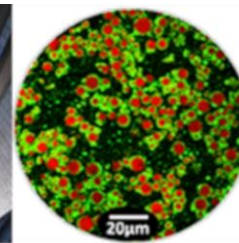
➤ *Propionibacterium freudenreichii*-containing cheese: anti-colitis effects demonstrated *in vivo*

Colitis

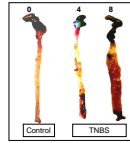
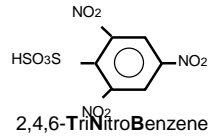
- GIT Chronic inflammation



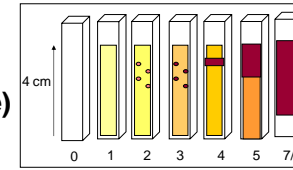
Ulcerative
Colitis



➤ First, try a single-strain experimental cheese



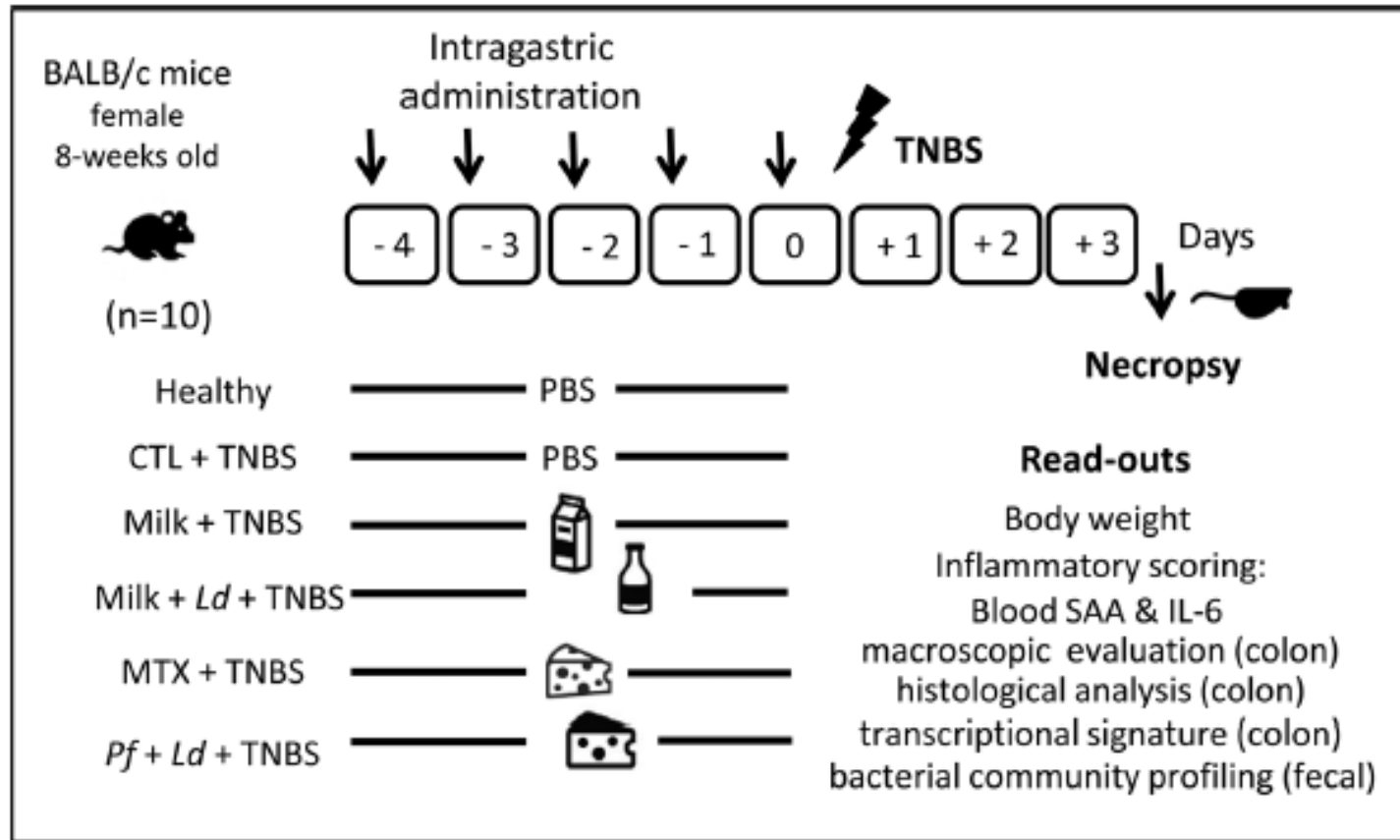
Macroscopic score (Wallace)



Bruno Pot



Benoit Foligné



Microfiltr. Milk

↓
Powder 100 g/L

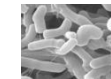


↓
Cream 150 g/L



↓
Sterilisation

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



↓
Pre-cheese

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

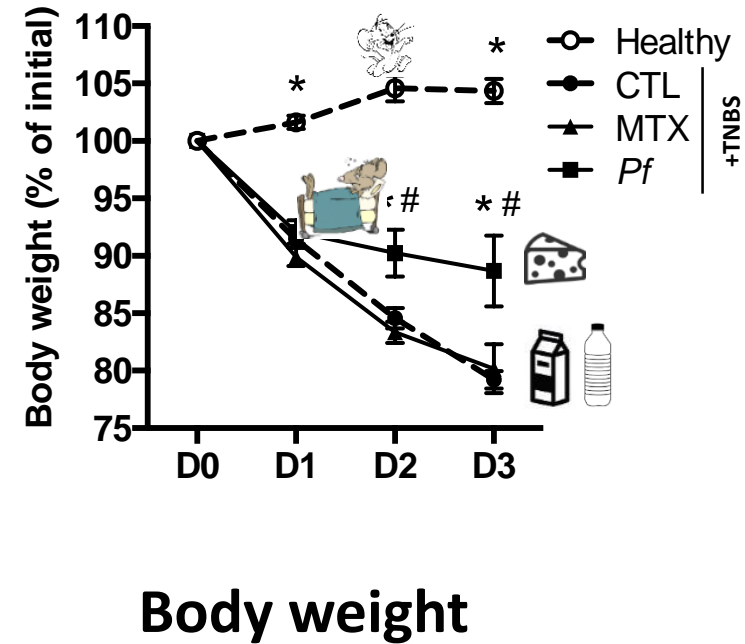
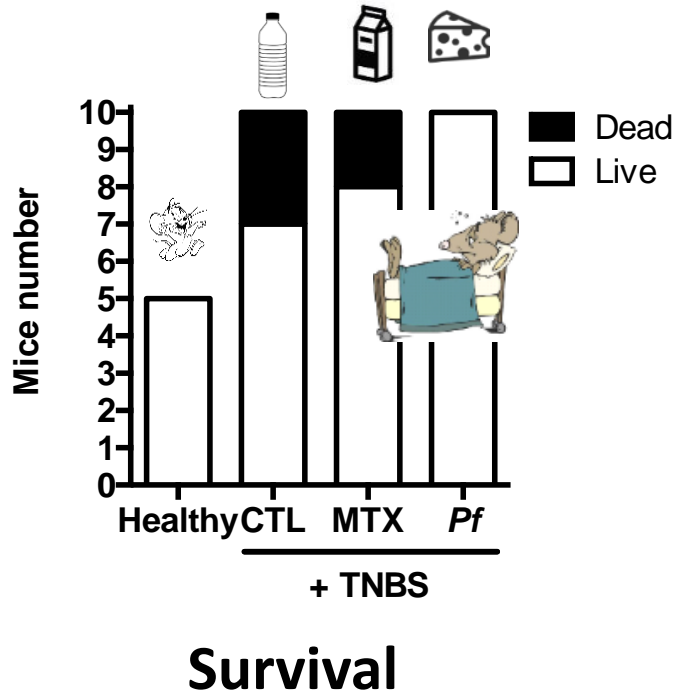


INRAE

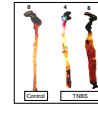
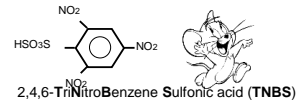
Propionibacteria immunomodulation
Fat matters



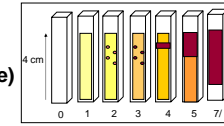
➤ Cheese prevents TNBS-induced colitis in mice



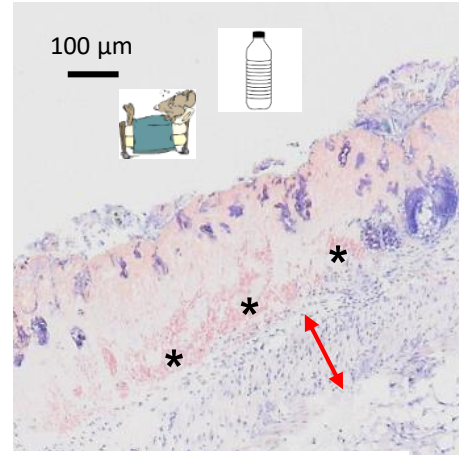
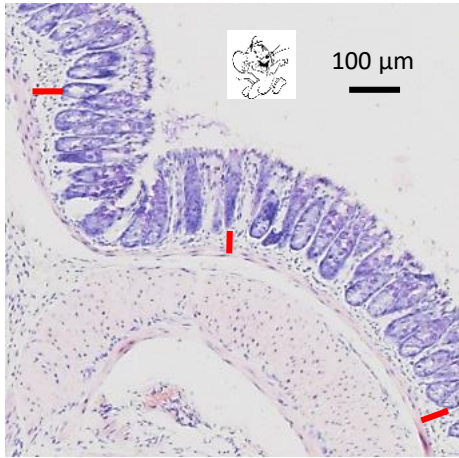
➤ Cheese prevents TNBS-induced colitis in mice



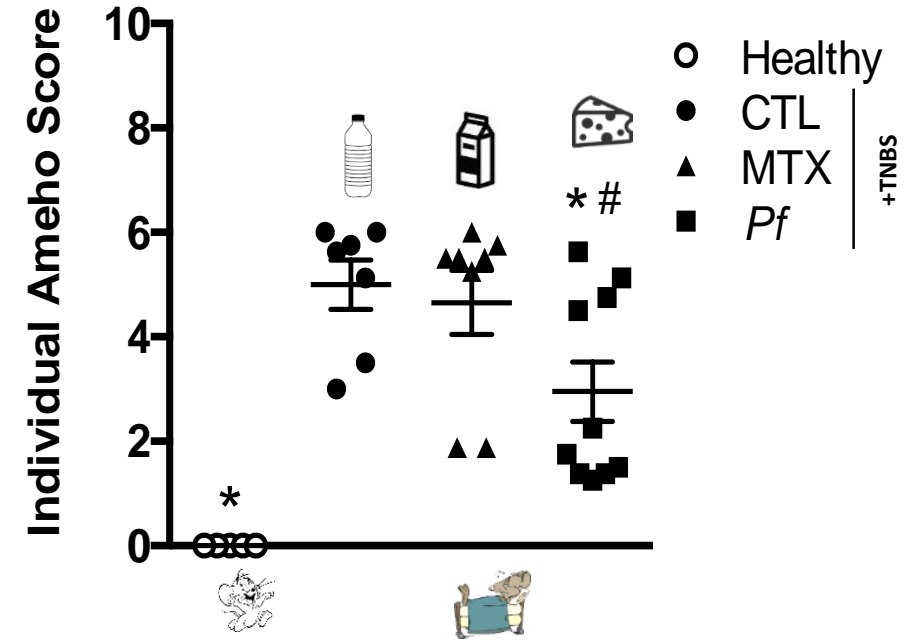
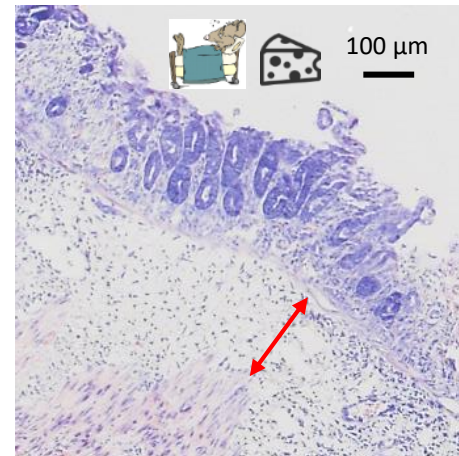
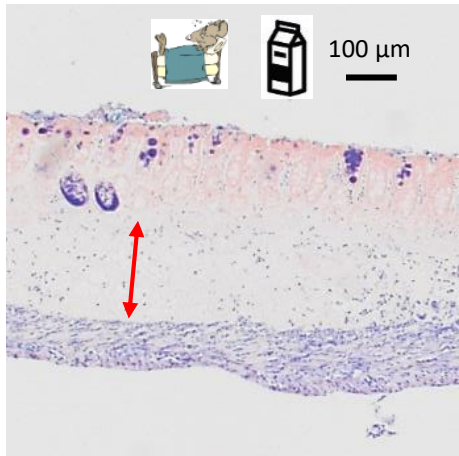
Macroscopic score (Wallace)



Bruno Pot



Benoit Foligné



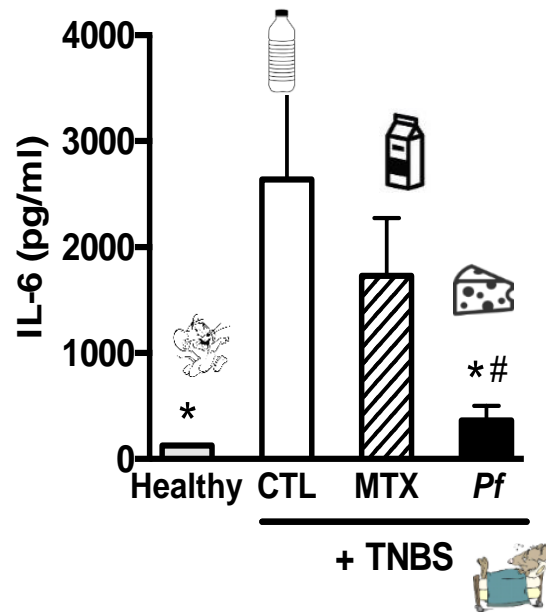
INRAE

Propionibacteria immunomodulation
Fat matters

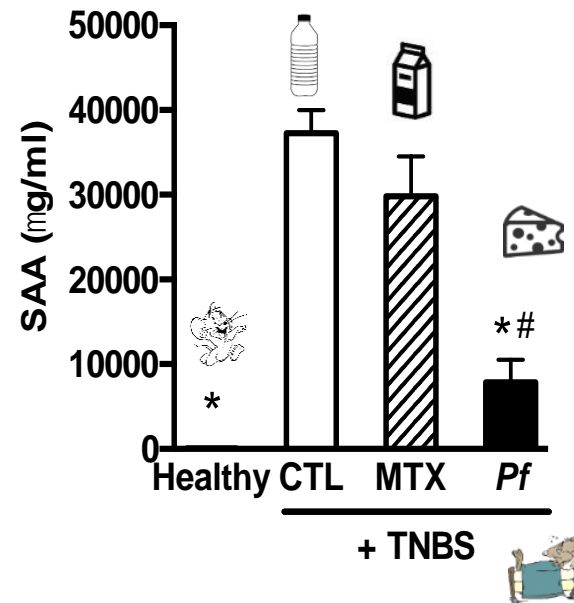


➤ Cheese prevents TNBS-induced colitis in mice

Blood inflammation markers



Interleukin 6



Serum Amyloid A



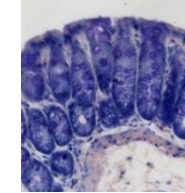
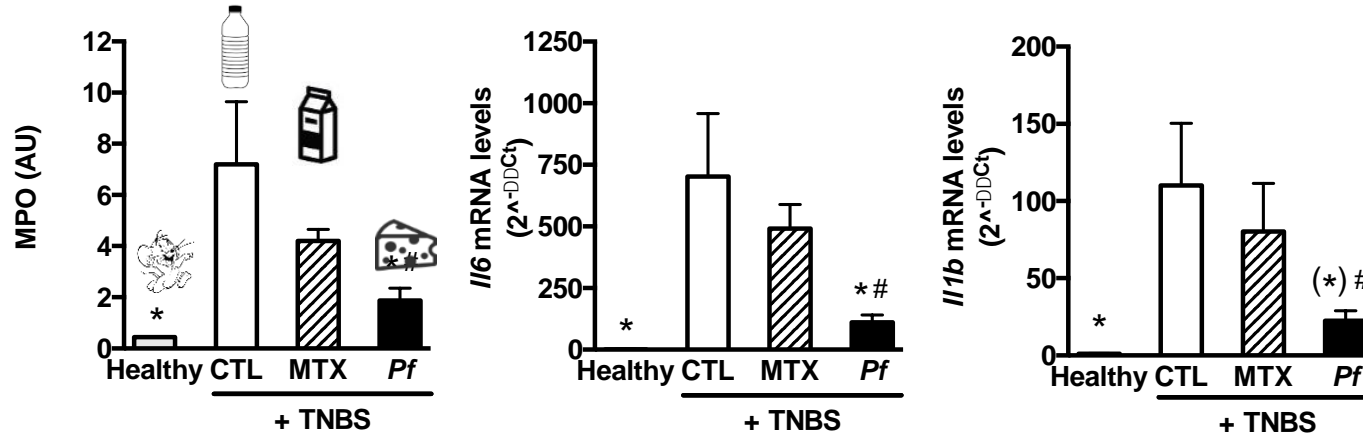
INRAE

Propionibacteria immunomodulation
Fat matters

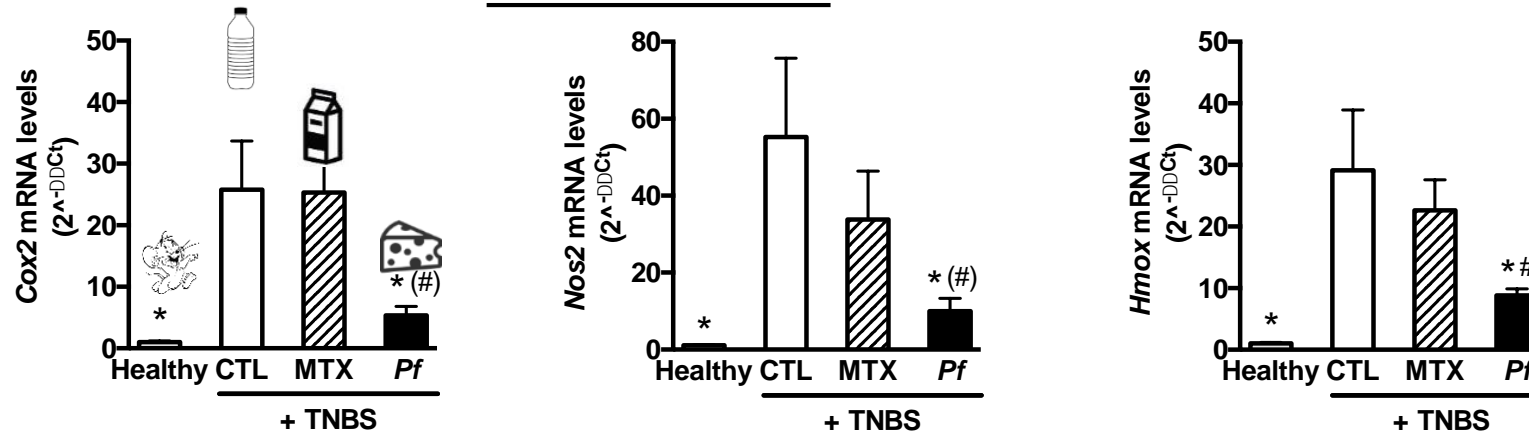


➤ Cheese prevents TNBS-induced colitis in mice

Colonic inflammatory markers



Colonic oxidative stress markers



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



INRAE

Propionibacteria immunomodulation
Fat matters

CIIL
CENTER FOR INFECTION
& IMMUNITY OF LILLE

STLO

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

L'INSTITUT
agro

➤ Then make a real Emmental cheese

Streptococcus thermophilus
&
Lactobacillus helveticus

Propionibacterium freudenreichii



Raw milk (1000 L)

Thermisation (90°C, 5 min)

Cooling down to 37°C

Addition of rennet

Addition of Lactic Acid Bacteria

Addition of Propionic Acid Bacteria

Clotting (coagulation)

Cutting of the curd

Cooking 55°C, 1h

Moulding, pressing, cooling down takes 24h

Salting

Cold room

Warm room

Ripening (weeks....to years!)



INRAE

Propionibacteria immunomodulation
Fat matters

STLO



L'INSTITUT
agro

Emmental: prevention of DSS-induced

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

C57BL6
 8th week age



1st Day



5th Day

12th Day

Intragastric gavage

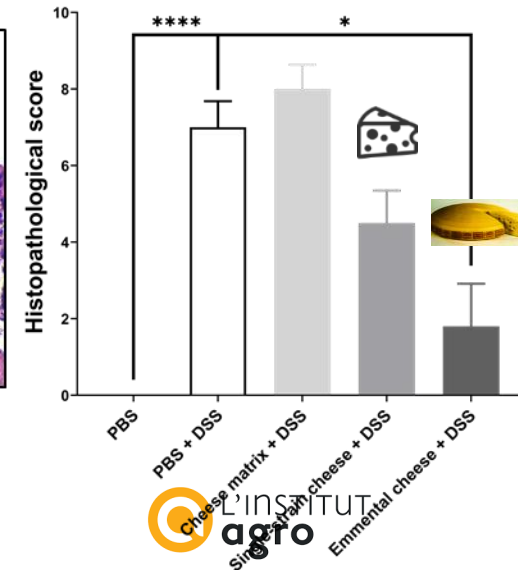
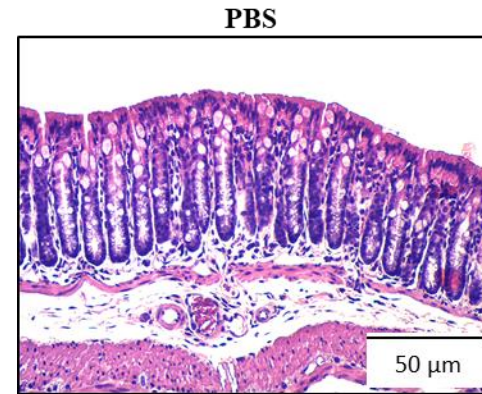
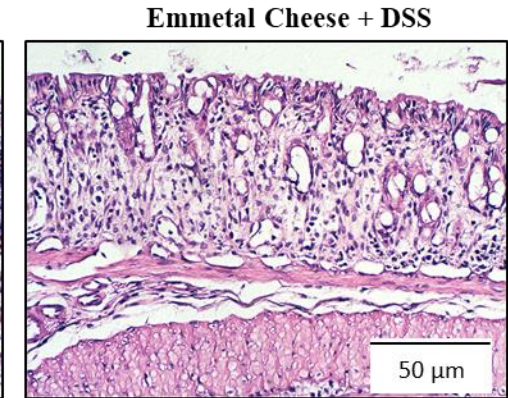
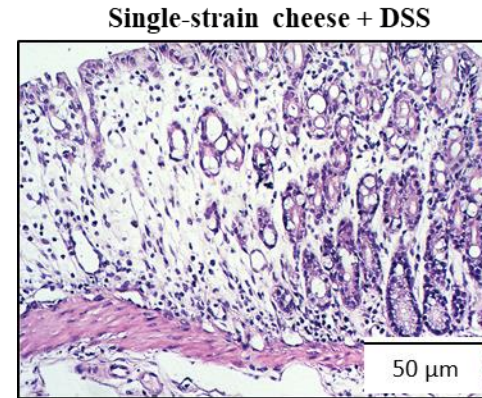
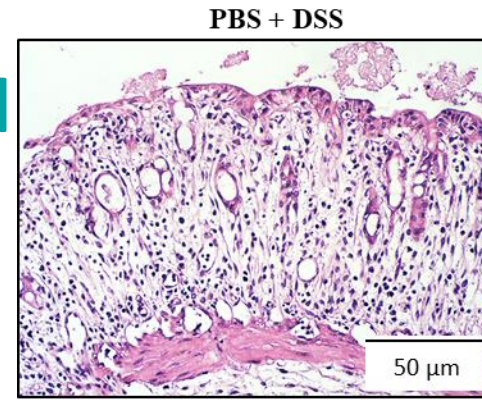
Gavage 500 μ L
 (400 mg cheese in
 500 μ L PBS pH 7.4)

3% (w/v) DSS solution
 (36–50 25 kDa)

Colitis induction

Beginning

Euthanasia



Vasco Azevedo



INRAE

Propionibacteria immunomodulation
 Fat matters



STLO



L'INSTITUT
 agro
 Emmental cheese + DSS

➤ What is the respective role of the different constituents of milk?

➤ Lipids ?

➤ Proteins ?

➤ Aqueous phase ?



➤ Skim milk permeate, skim milk, Whole milk

➤ Fermented by Pf 129 until 9 logs reached

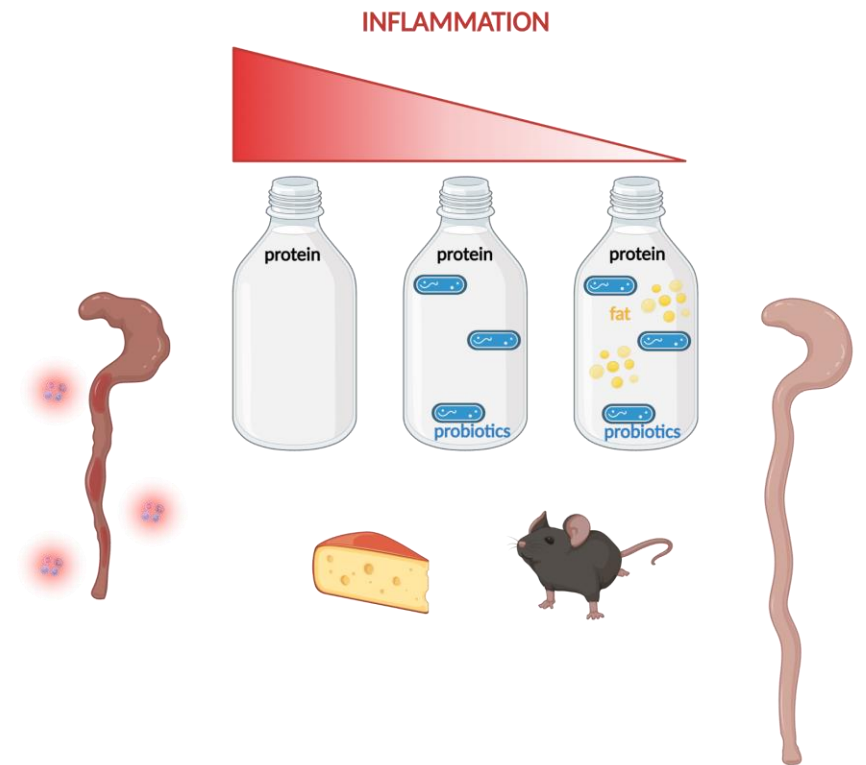
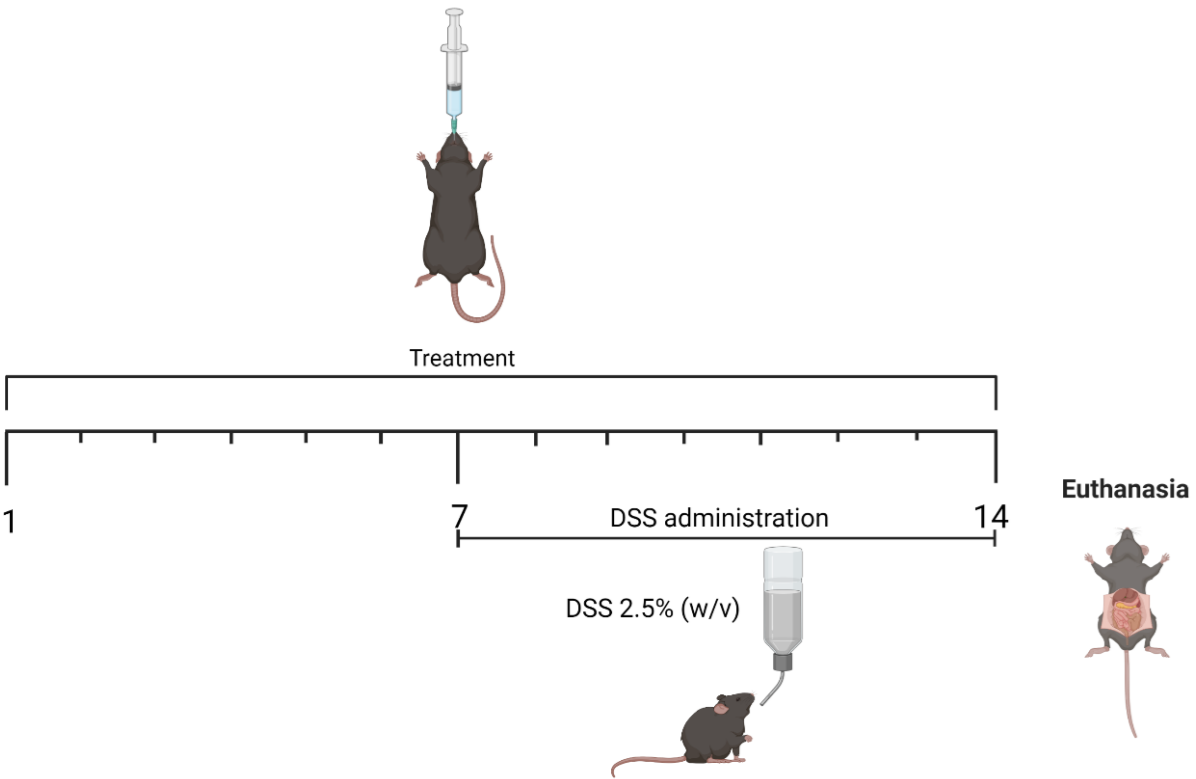


INRAE

Propionibacteria immunomodulation
Fat matters



➤ Products given by gavage before colitis induction in mice



INRAE

Propionibacteria immunomodulation

Fat matters

UFMG
UNIVERSIDADE FEDERAL
DE MINAS GERAIS

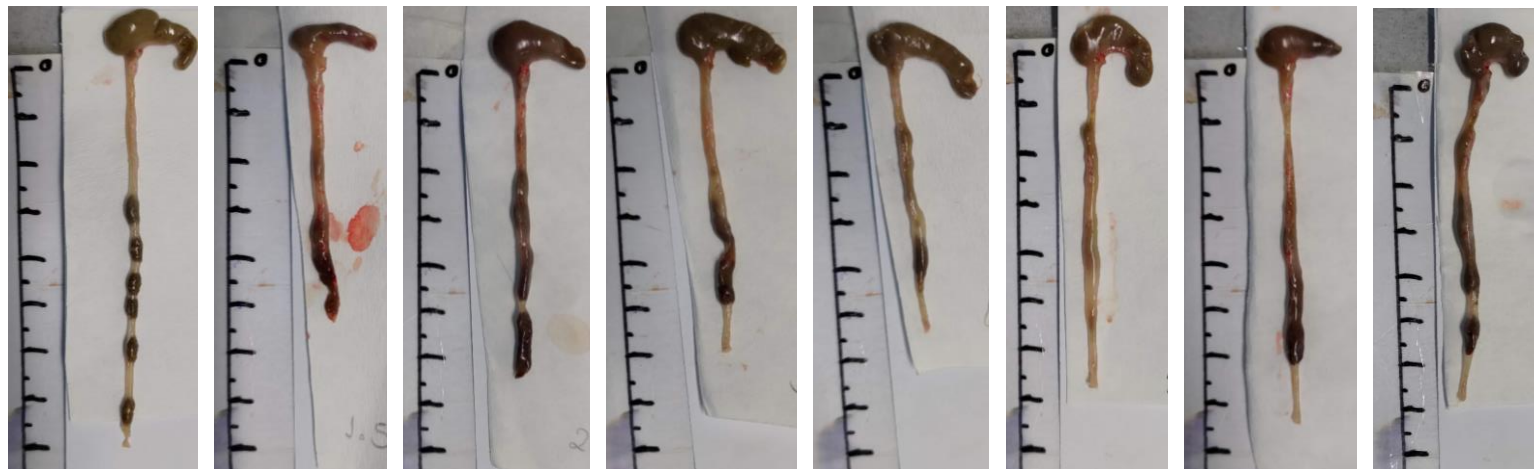
LGCM
Laboratório de Genética
Celular e Molecular

STLO

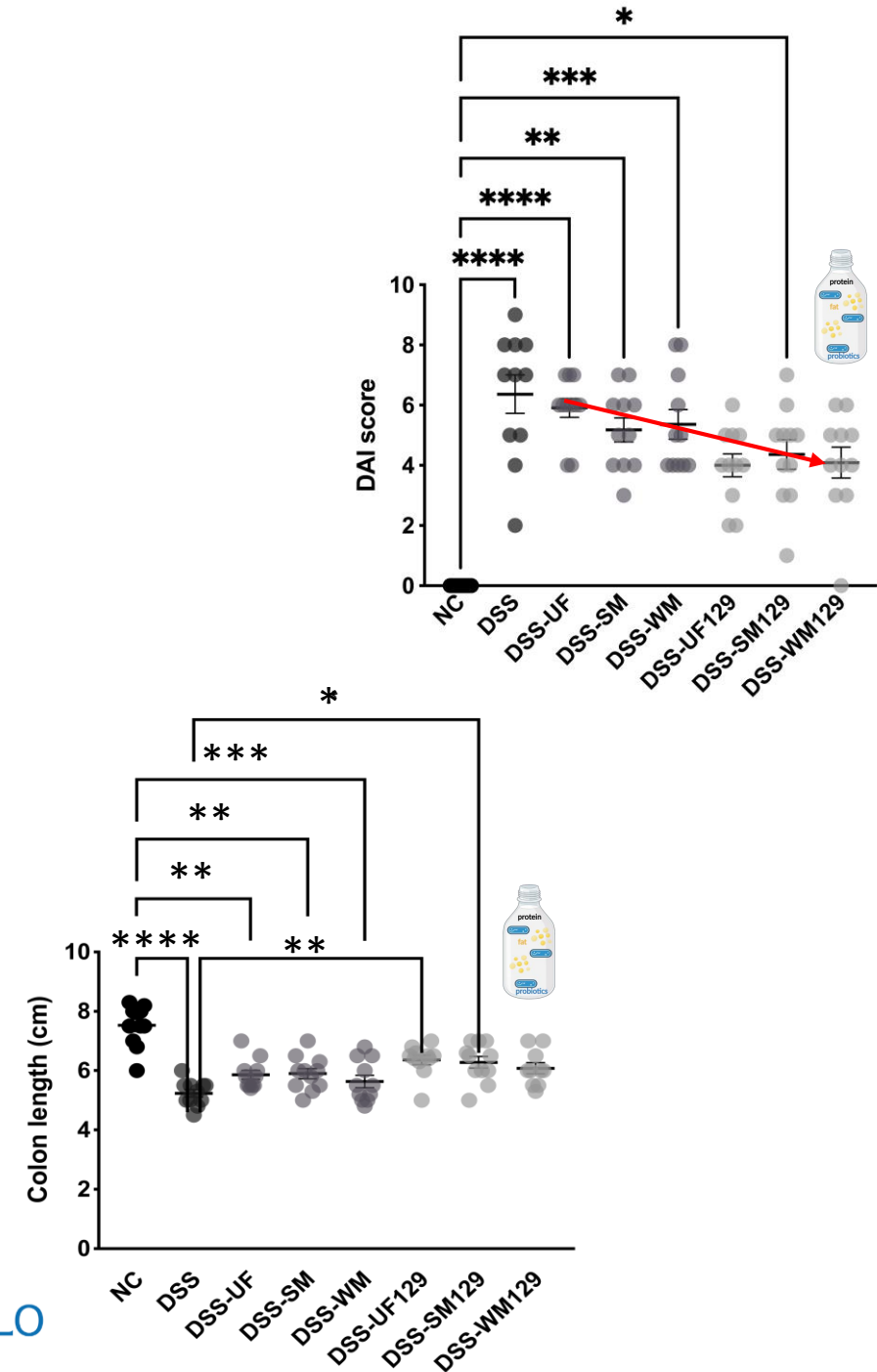


L'INSTITUT
agro

➤ Effect on colon and DAI



NC DSS DSS-UF DSS-SM DSS-WM DSS-UF 129 DSS-SM 129 DSS-WM 129



INRAE

Propionibacteria immunomodulation

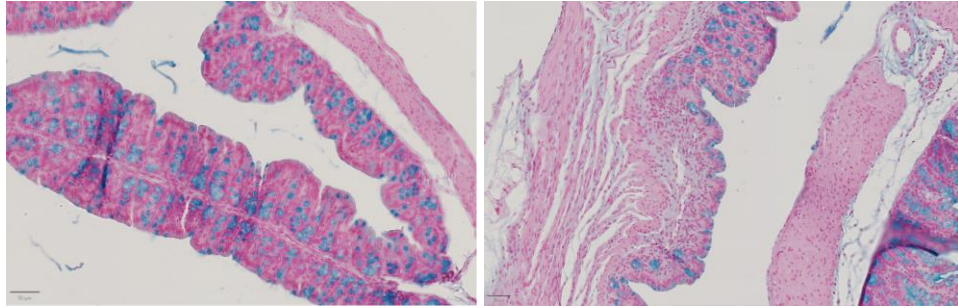
Fat matters

UFMG
UNIVERSIDADE FEDERAL DE MINAS GERAIS

LGCM
Laboratório de Genética Celular e Molecular

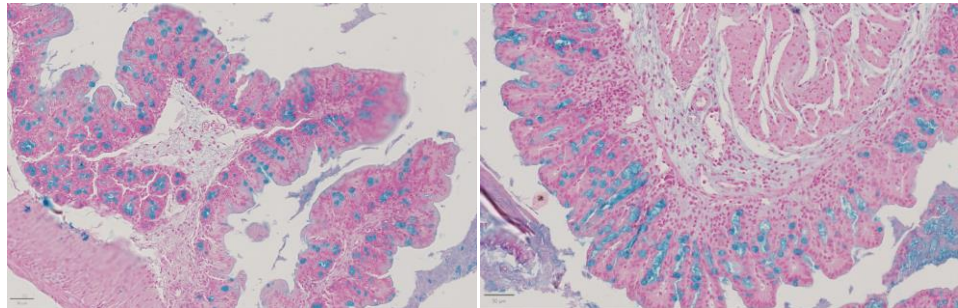
STLO

➤ Only fermented whole milk protects the colon mucosa architecture



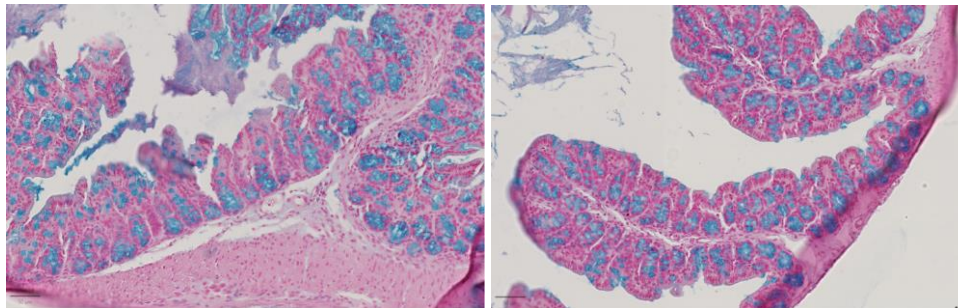
NC

DSS



DSS-WM

DSS-UF129



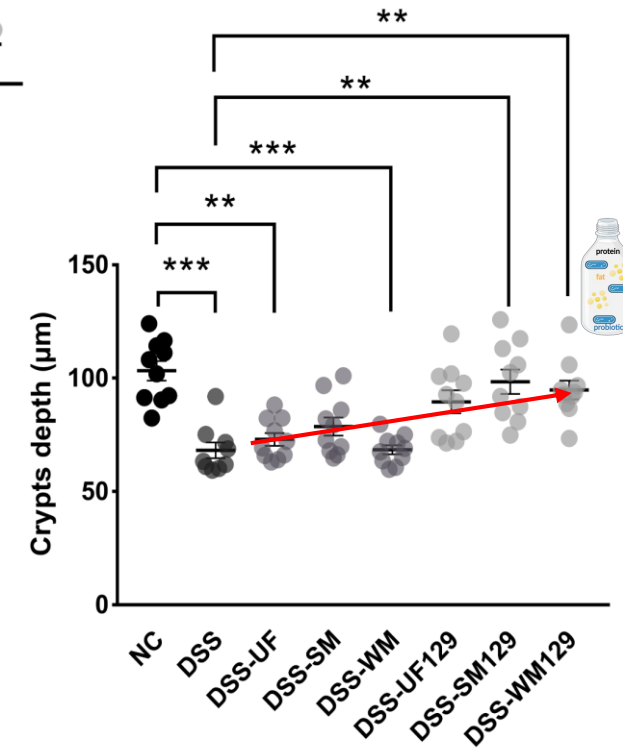
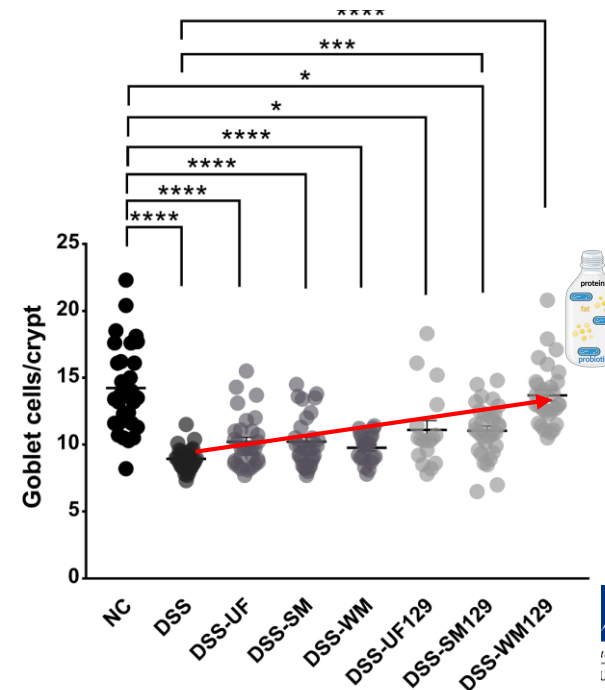
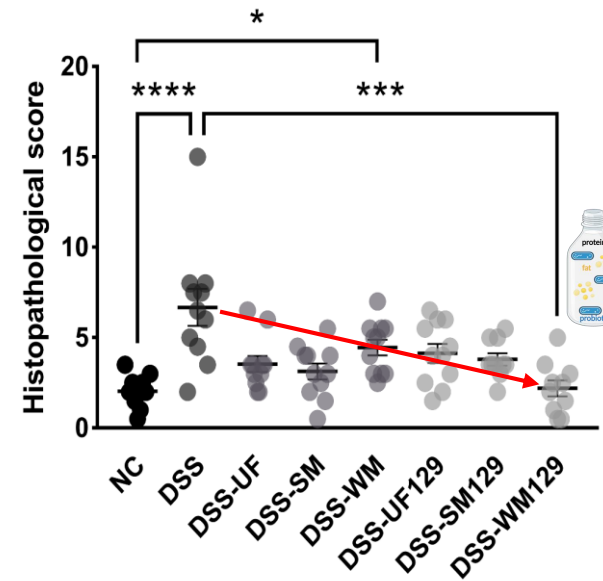
DSS-SM129

DSS-WM129

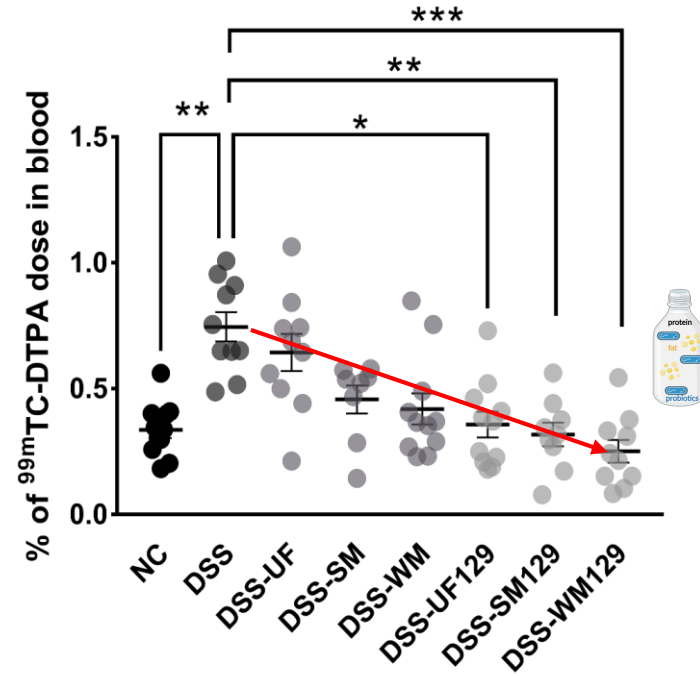
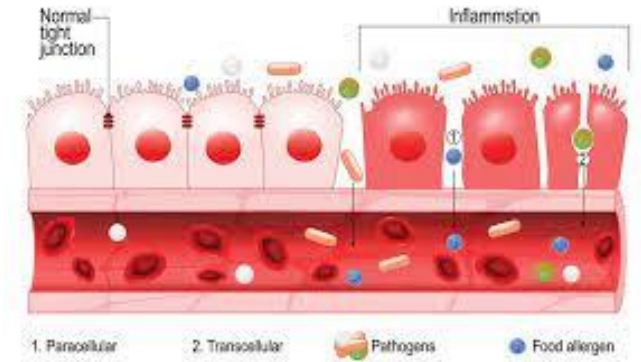
INRAE

Propionibacteria immunomodulation

Fat matters



➤ Gut barrier permeability

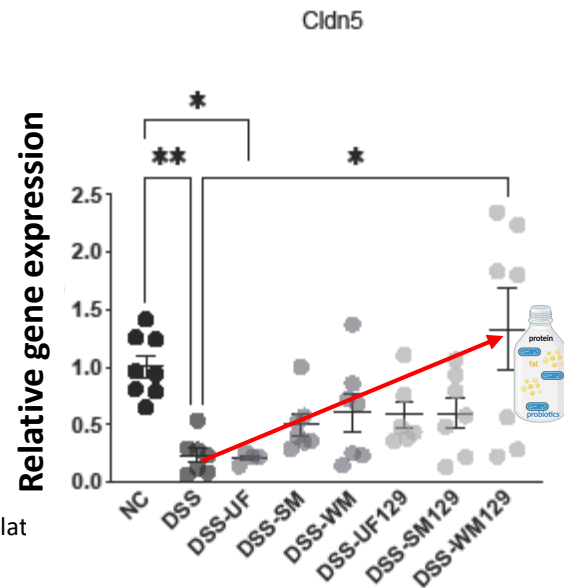
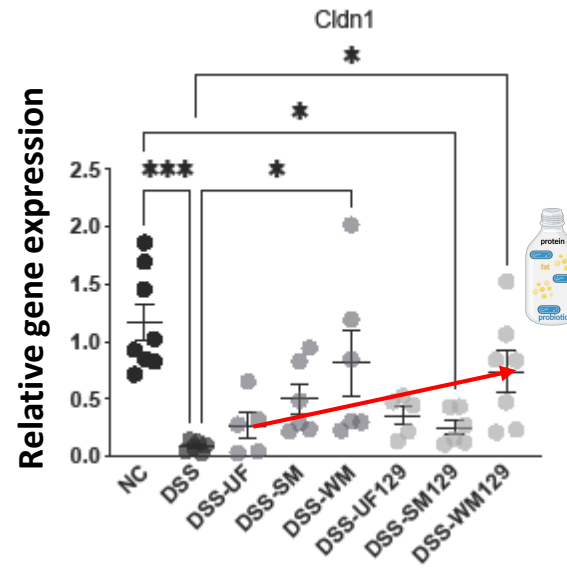
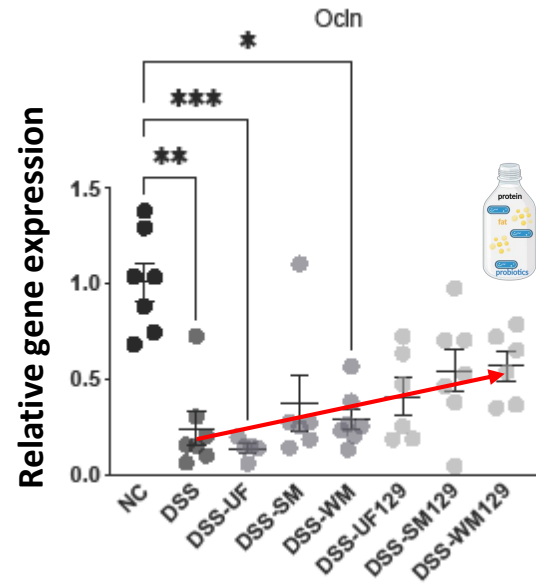


INRAE

Propionibacteria immunomodulation
Fat matters



➤ Gene expression



Relative gene expression

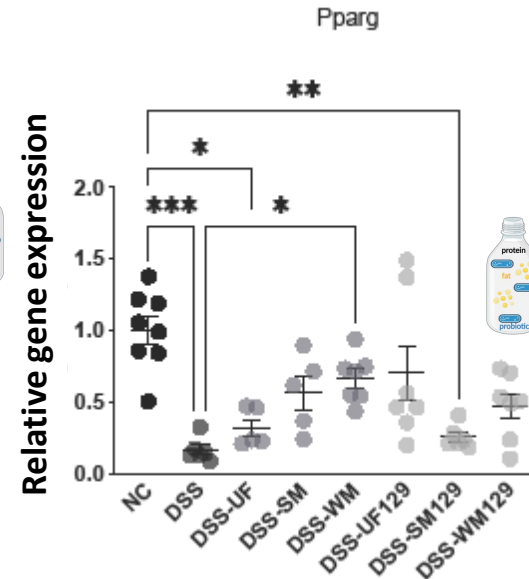
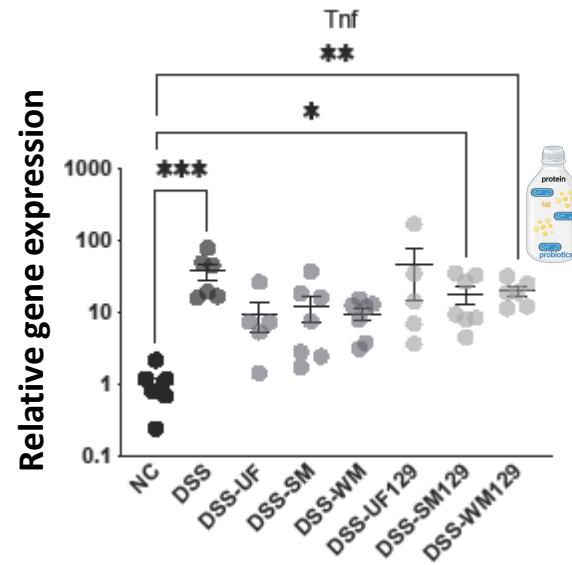
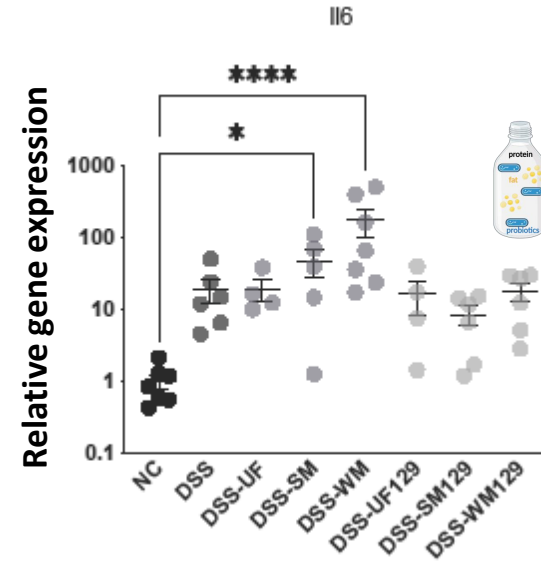
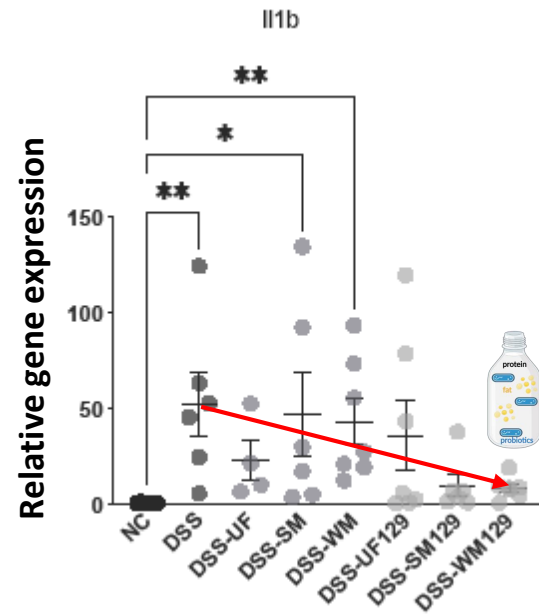


INRAE

Propionibacteria immunomodulat
Fat matters

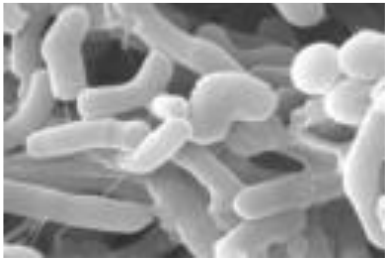


➤ Gene expression



- Propionibacteria immunomodulatory probiotics
 - Fermented dairy products, good probiotic delivery vehicle
 - Dairy fat plays a role

➤ Thank you for your attention!
Any kind question?



INRAE

Propionibacteria immunomodulation
Fat matters

