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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.

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

Gwénaél JAN

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<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!)

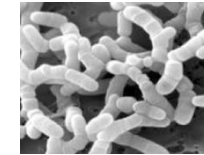


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Propionibacteria immunomodulation
Fat matters



- 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!!

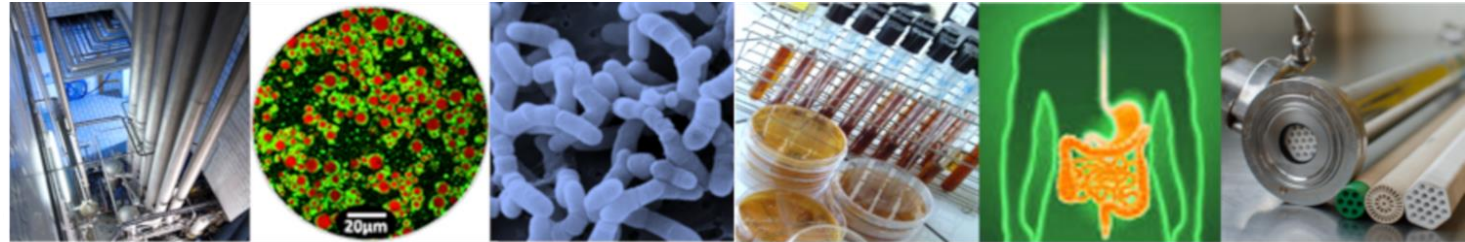


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Propionibacteria immunomodulation
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➤ *Propionibacterium freudenreichii*: Who's that bug?

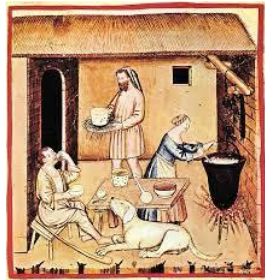


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Fat matters



➤ Emmental and other cheeses with holes: a long story



Flavour:

Fruity

Sour

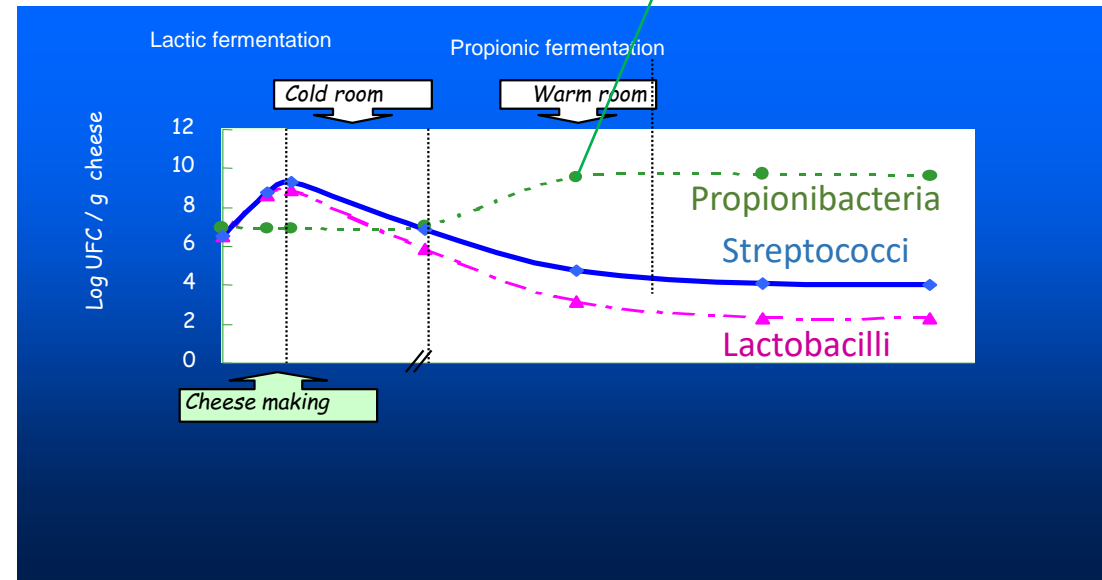
Picante

Propionate

Acetate

CO₂

...Esters



➔ The Fitz equation (1878)



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

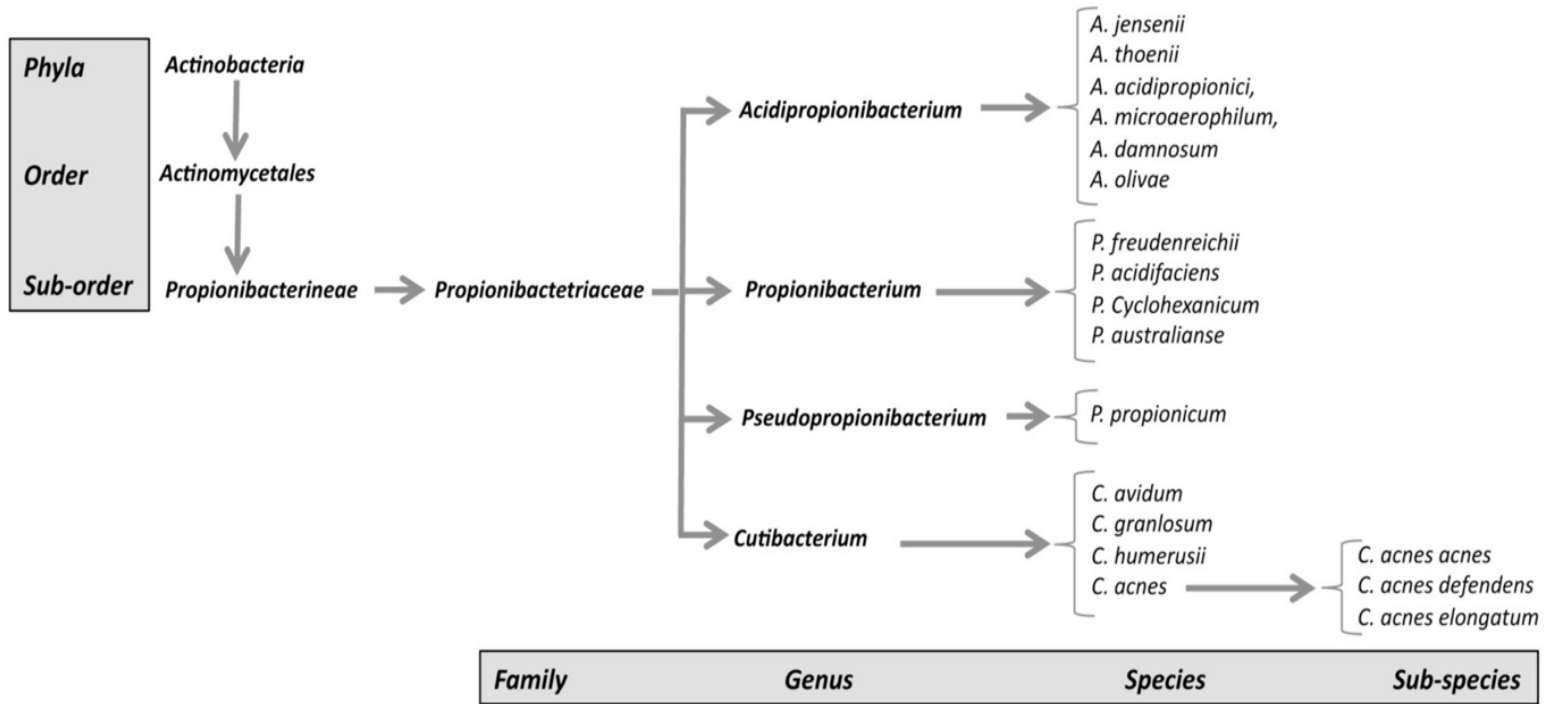


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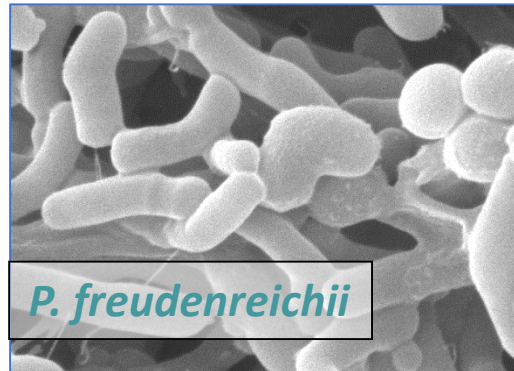
Propionibacteria immunomodulation
Fat matters



➤ Propionibacteria, in a few words



Gram +
 pleiomorphic
 anaerobic to microaérophilic
 non motiles et non sporulated

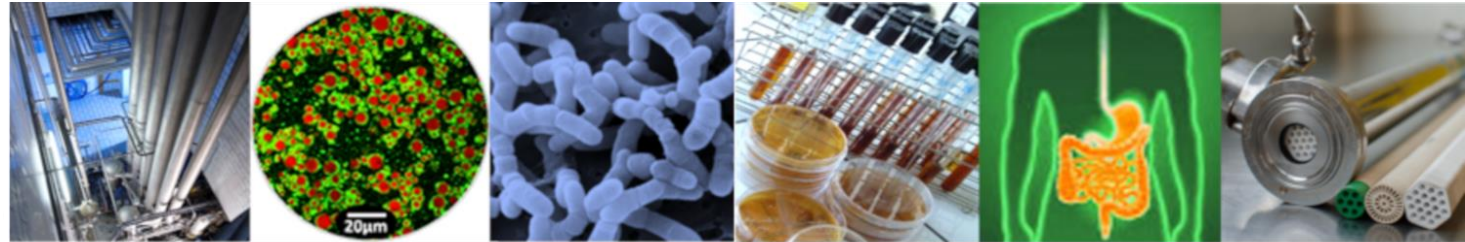


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 Fat matters



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



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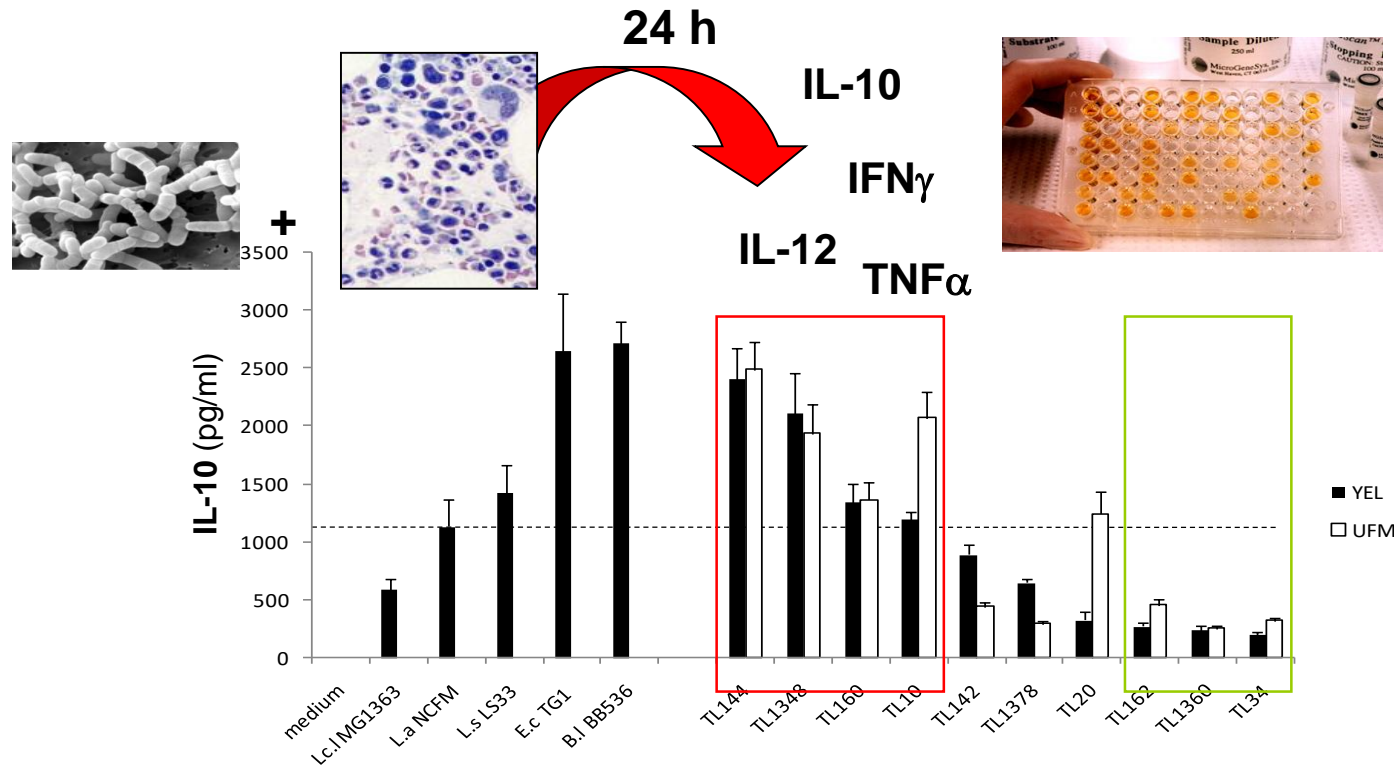
➤ Cytokine stimulation on human PBMCs (mononucleated leucocytes from donors)



Bruno Pot



Benoit Foligné



The best anti-inflammatory strains

The less anti-inflammatory strains



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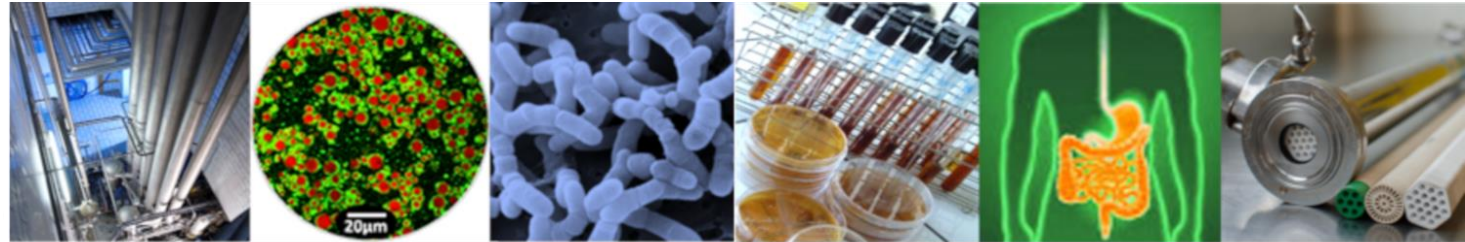
Propionibacteria immunomodulation
Fat matters



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

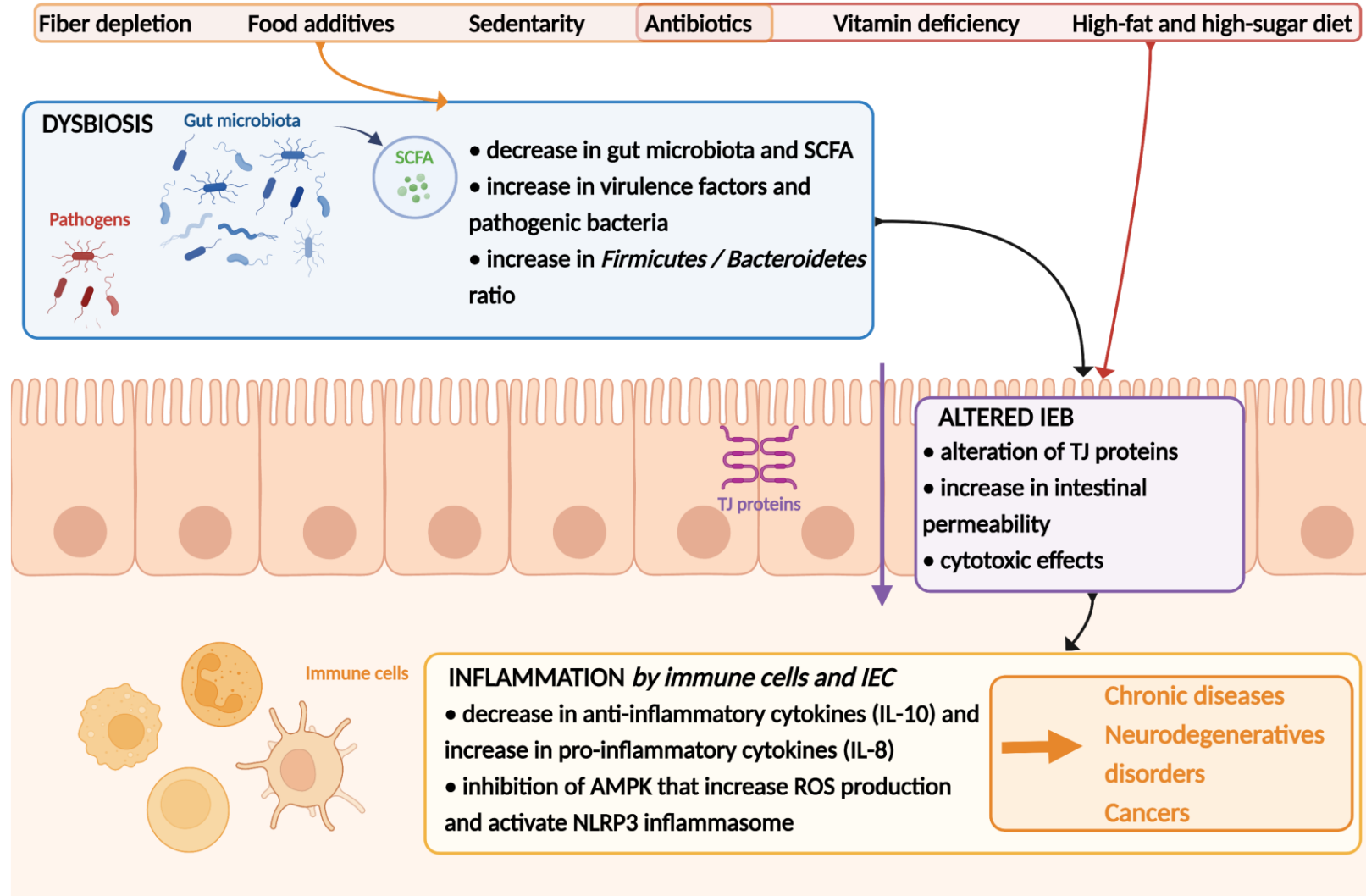


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Propionibacteria immunomodulation
Fat matters



> Inflammation of the gut : a growing concern



➤ Examples of digestive inflammatory diseases

Colitis

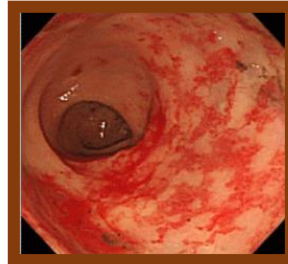
- GIT Chronic inflammation



Ulcerative Colitis



Mucositis



GI Mucositis



Oral mucositis

5-Fluoracil (5-FU)

- Head, neck and colon cancer.

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



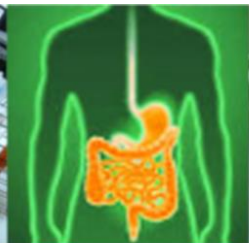
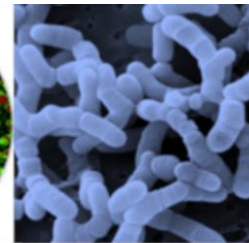
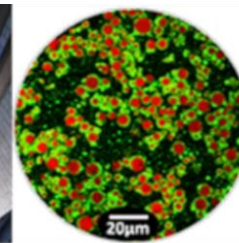
➤ *Propionibacterium freudenreichii*-fermented milk: anti-colitis effects demonstrated *in vivo*

Colitis

- GIT Chronic inflammation



Ulcerative Colitis



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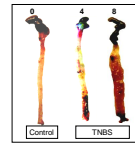
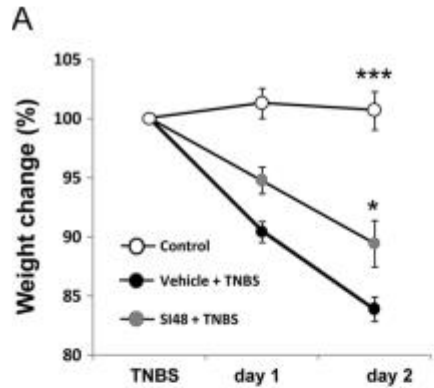
Propionibacteria immunomodulation
Fat matters

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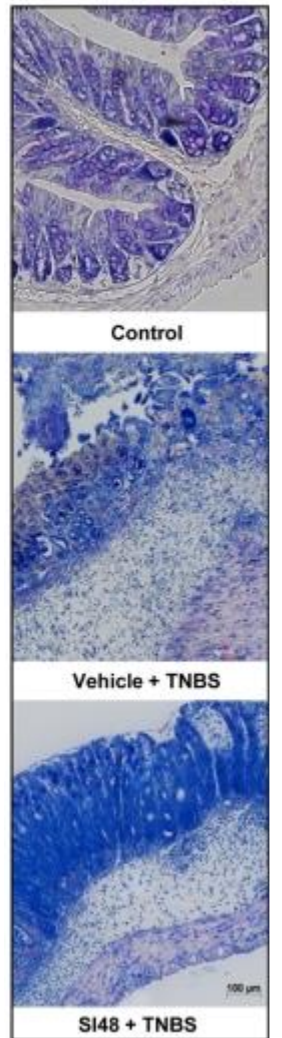
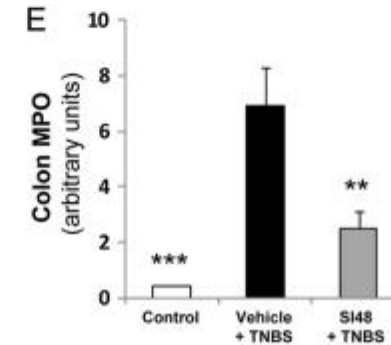
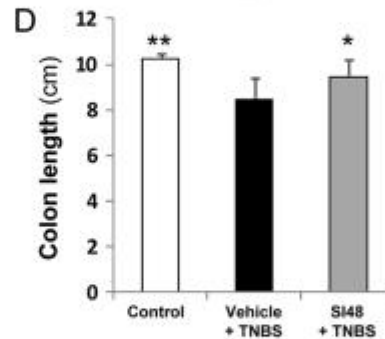
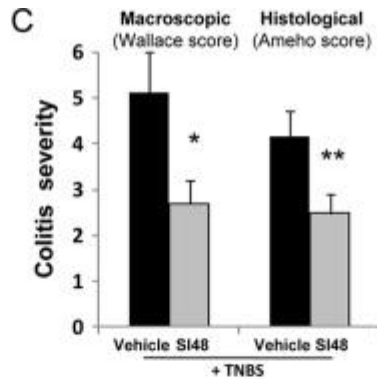
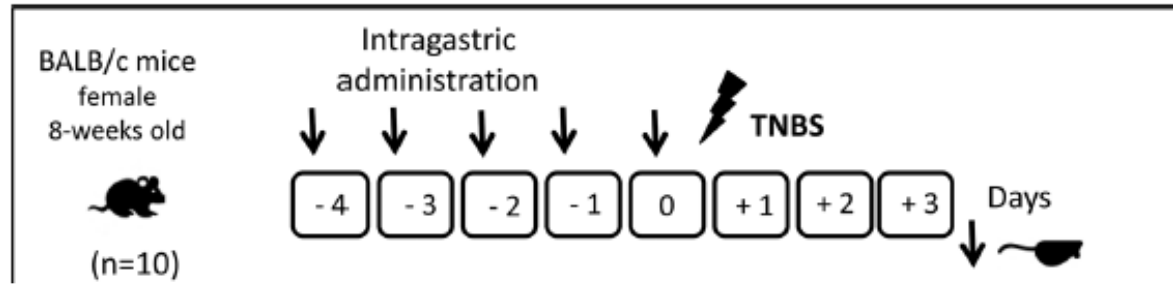
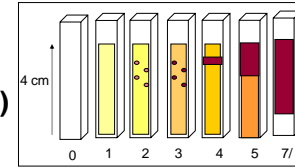


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➤ Propionibacteria: prevention of TNBS-induced colitis in mice



Macroscopic score (Wallace)



Benoit Foligné



Bruno Pot



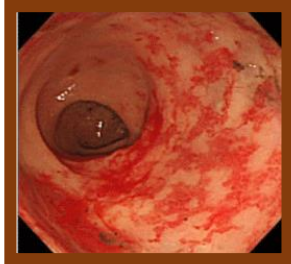
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Propionibacteria immunomodulation
Fat matters



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

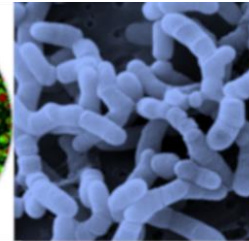
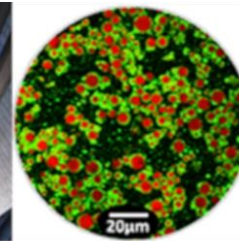
Mucositis



GI Mucositis



Oral mucositis



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- Head, neck and colon cancer.

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



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Propionibacteria immunomodulation

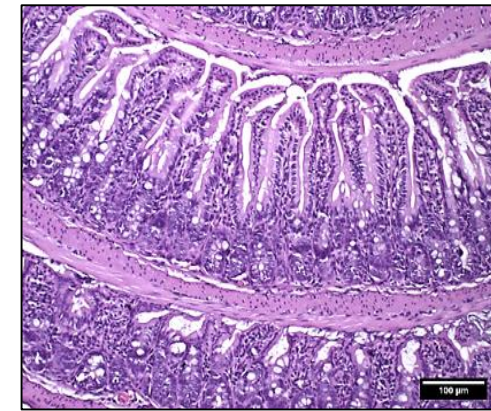
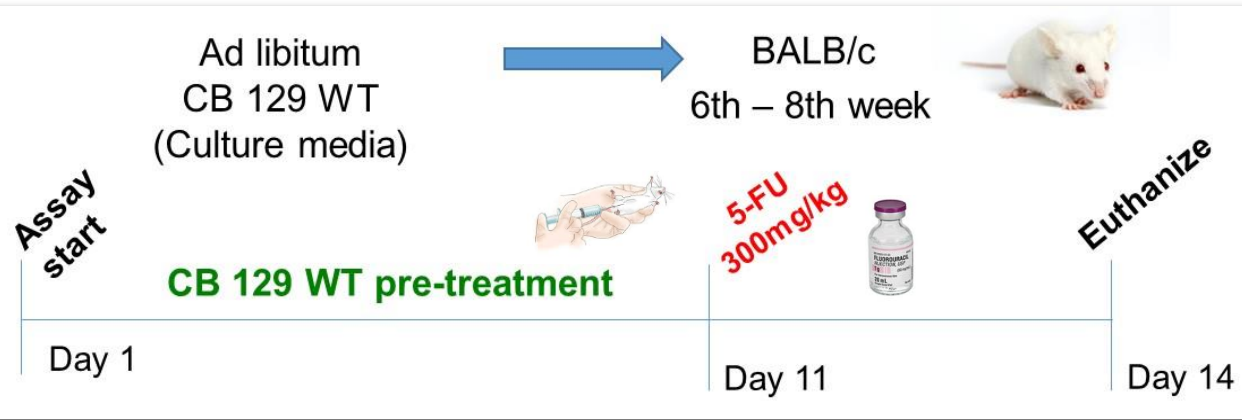
Fat matters

STLO

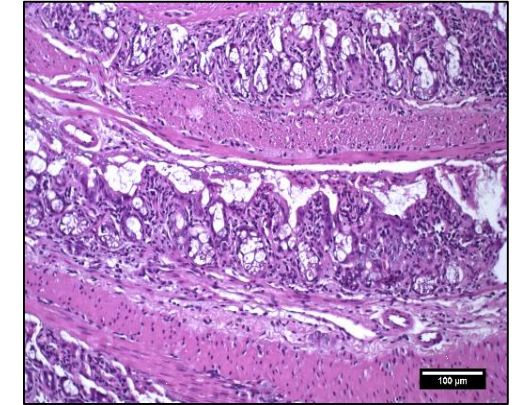
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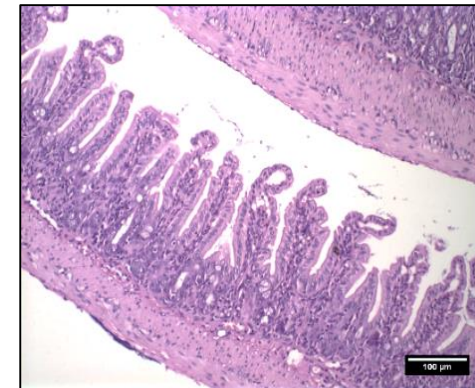
➤ Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice



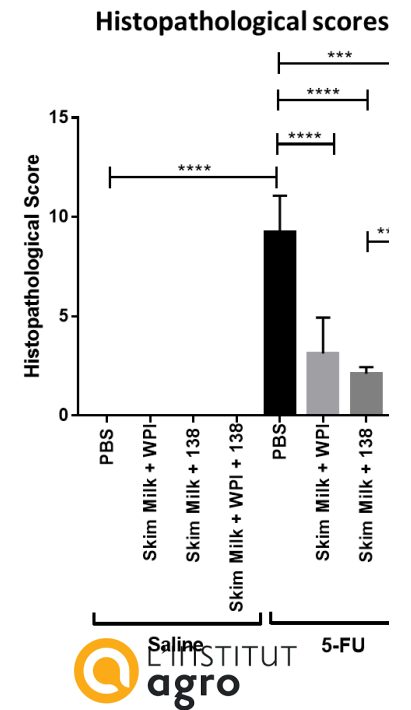
Healthy Ileum



PBS + 5-FU



Skim milk + propio



Vasco Azevedo



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Propionibacteria immunomodulation
Fat matters

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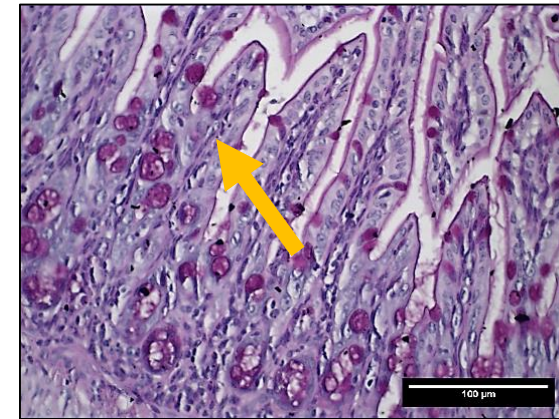
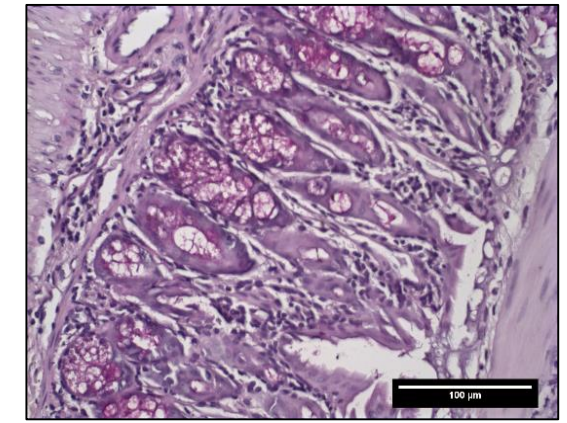
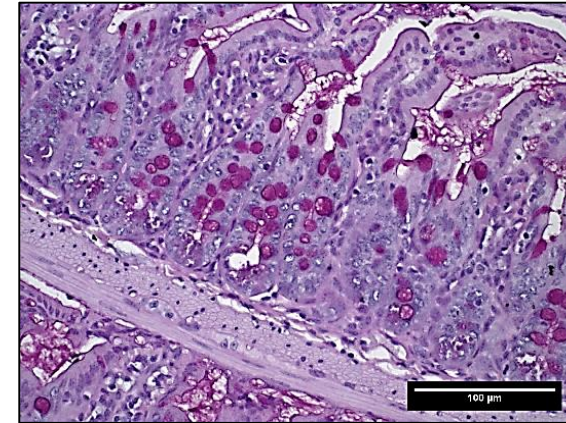
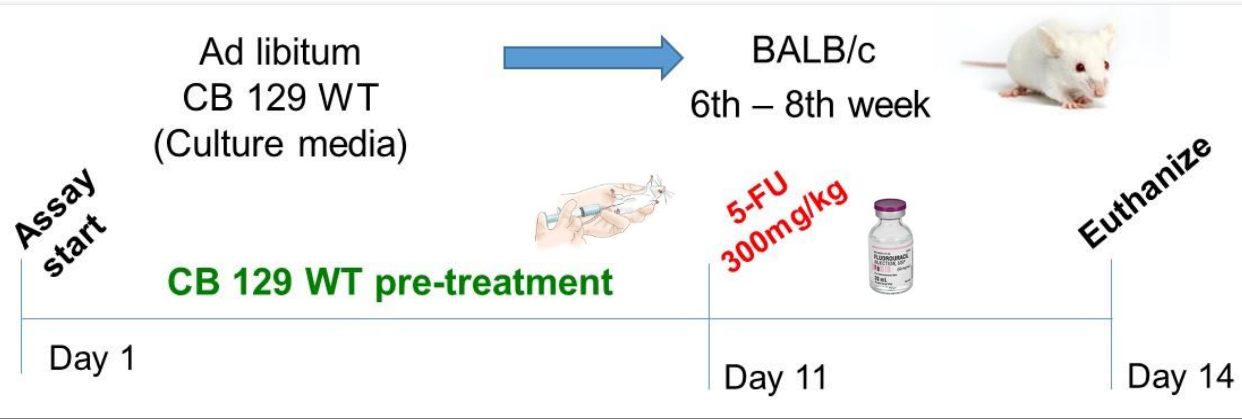
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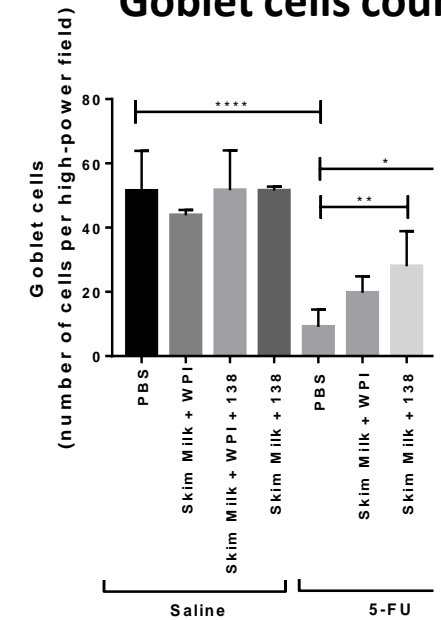
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Saline
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➤ Propionibacteria mitigate cancer chemotherapy-induced mucositis in mice



Goblet cells counts



Vasco Azevedo



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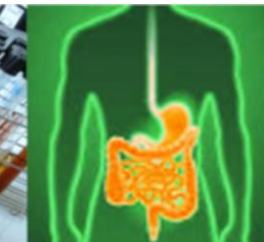
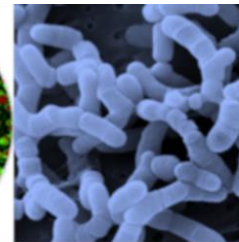
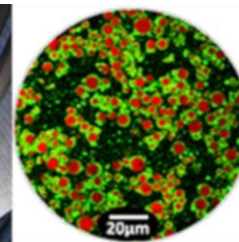
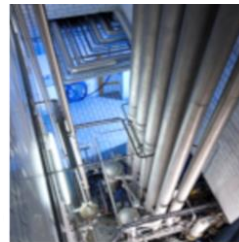
➤ *Propionibacterium freudenreichii*-containing cheese: anti-colitis effects demonstrated *in vivo*

Colitis

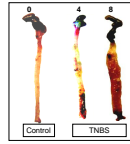
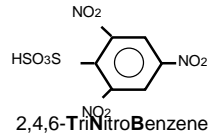
- GIT Chronic inflammation



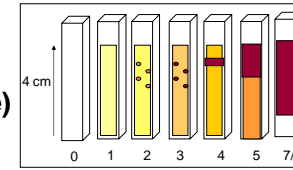
Ulcerative
Colitis



➤ First, try a single-strain experimental cheese



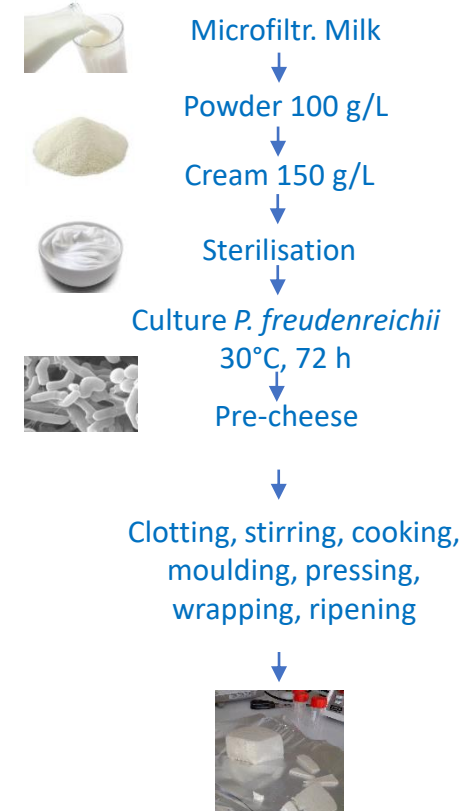
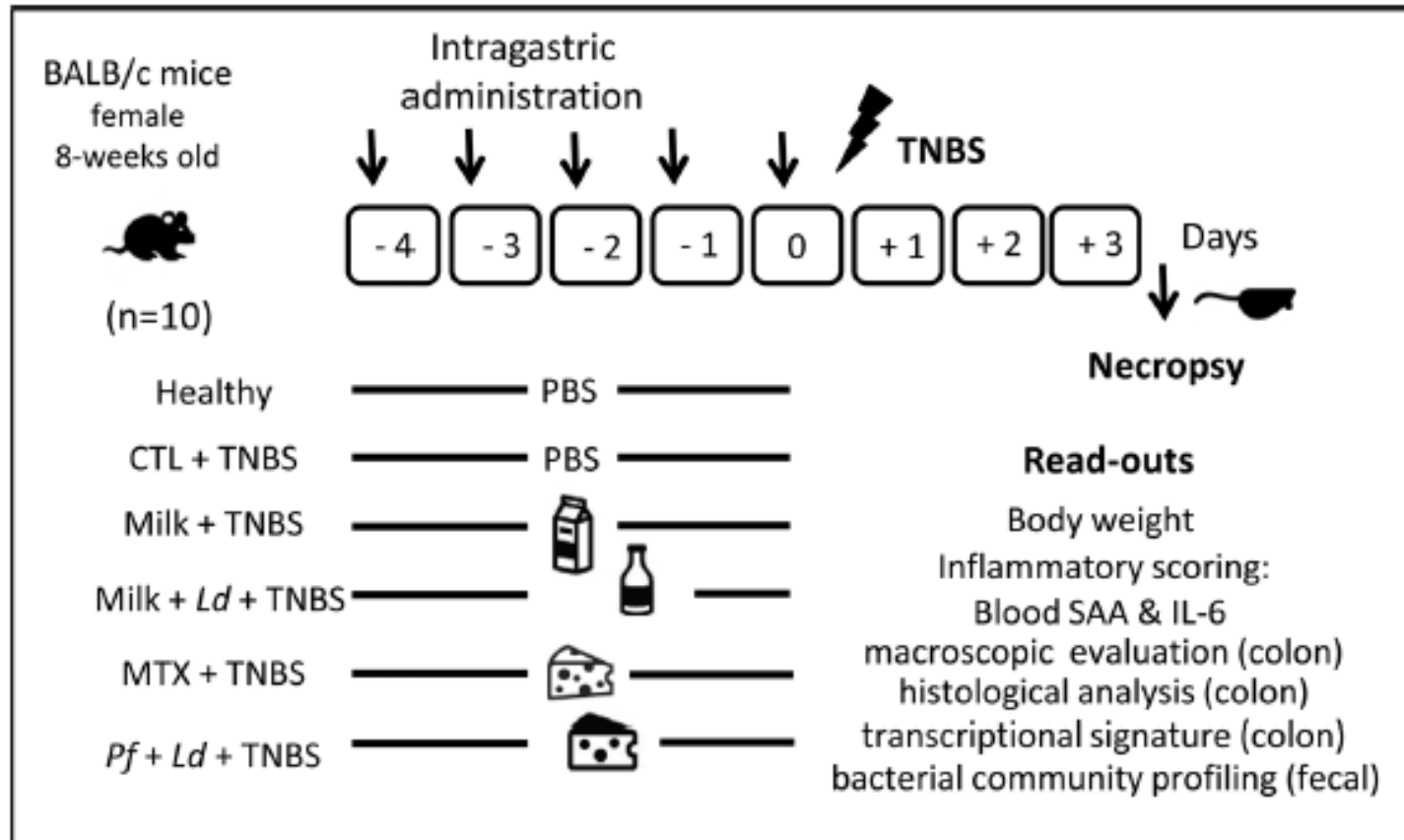
Macroscopic score (Wallace)



Bruno Pot



Benoit Foligné

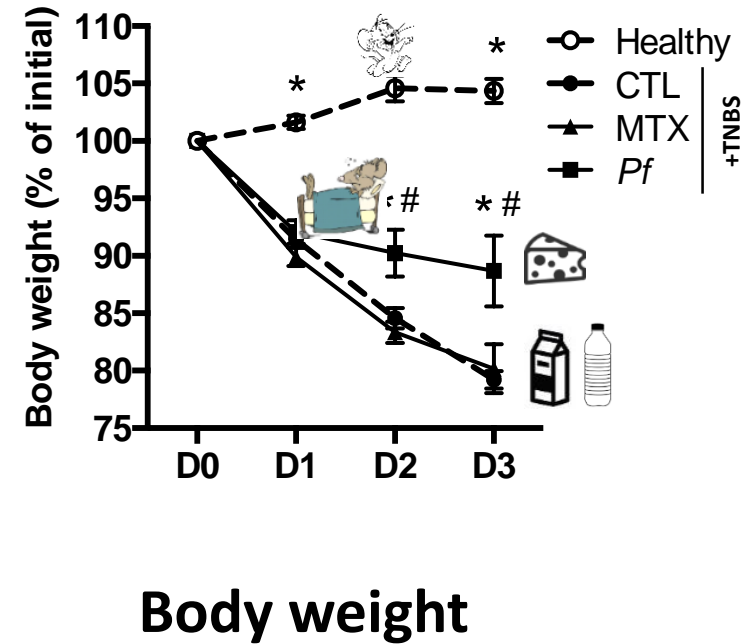
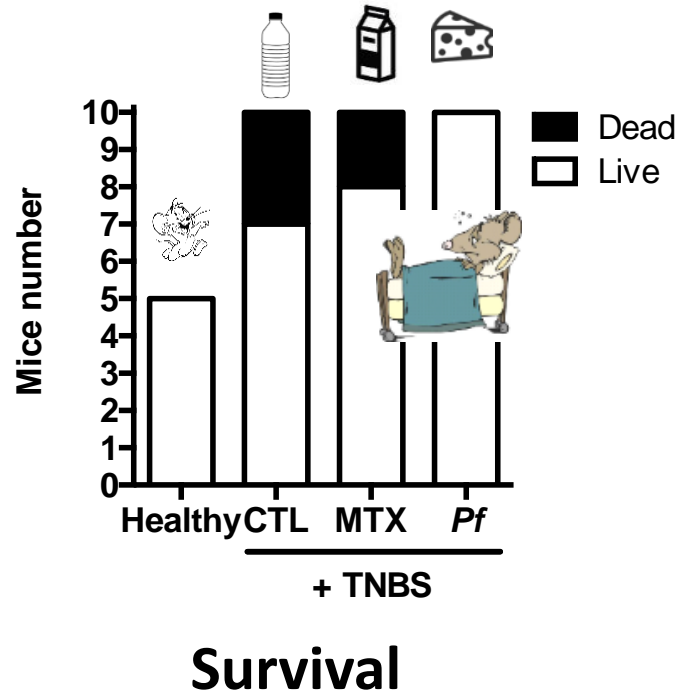


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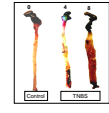
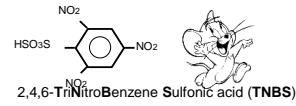
Propionibacteria immunomodulation
Fat matters



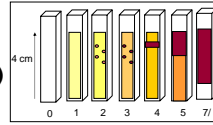
➤ Cheese prevents TNBS-induced colitis in mice



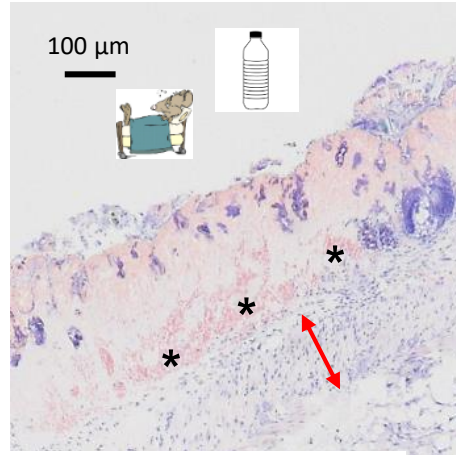
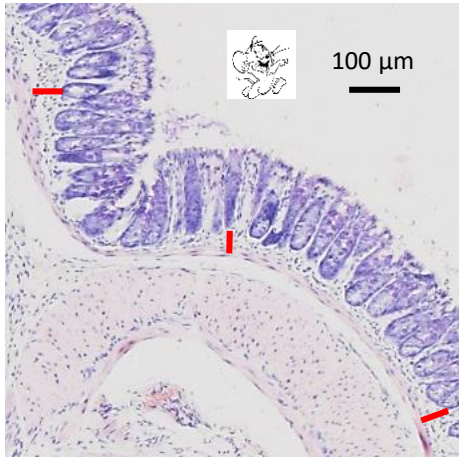
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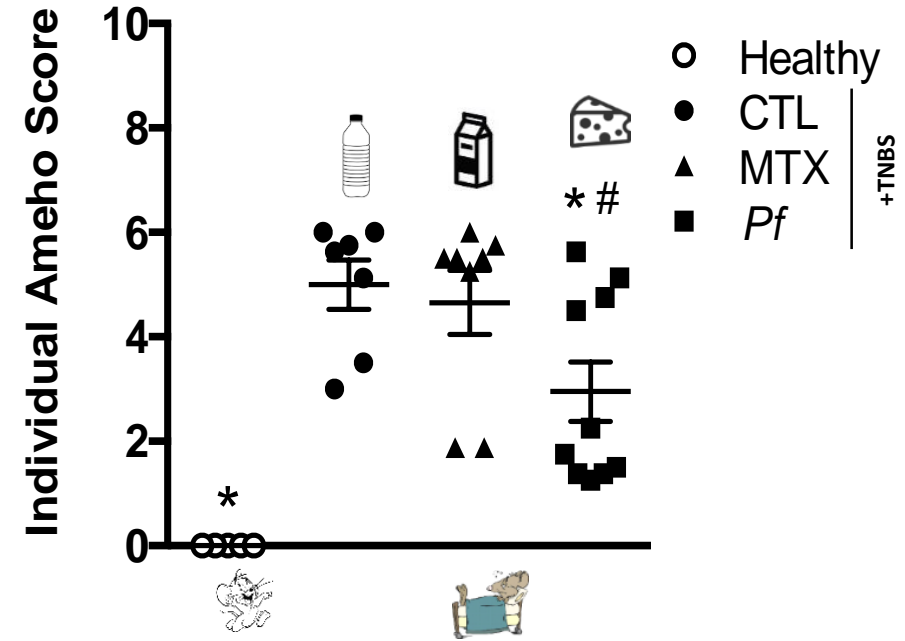
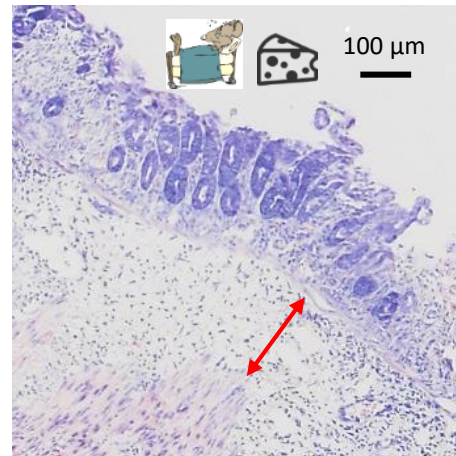
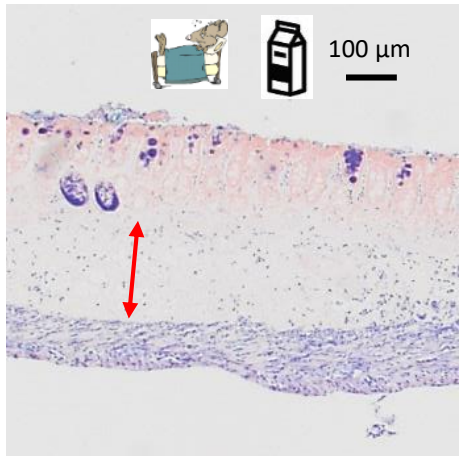
Macroscopic score (Wallace)



Bruno Pot



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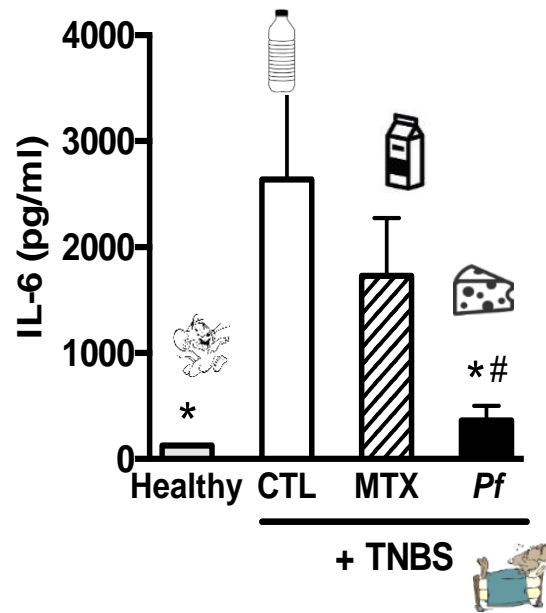
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Propionibacteria immunomodulation
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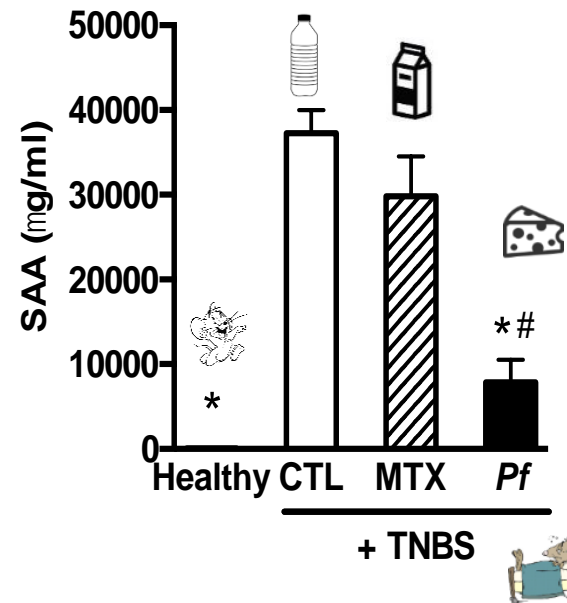


➤ Cheese prevents TNBS-induced colitis in mice

Blood inflammation markers



Interleukin 6



Serum Amyloid A



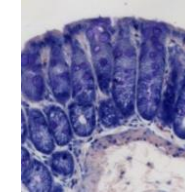
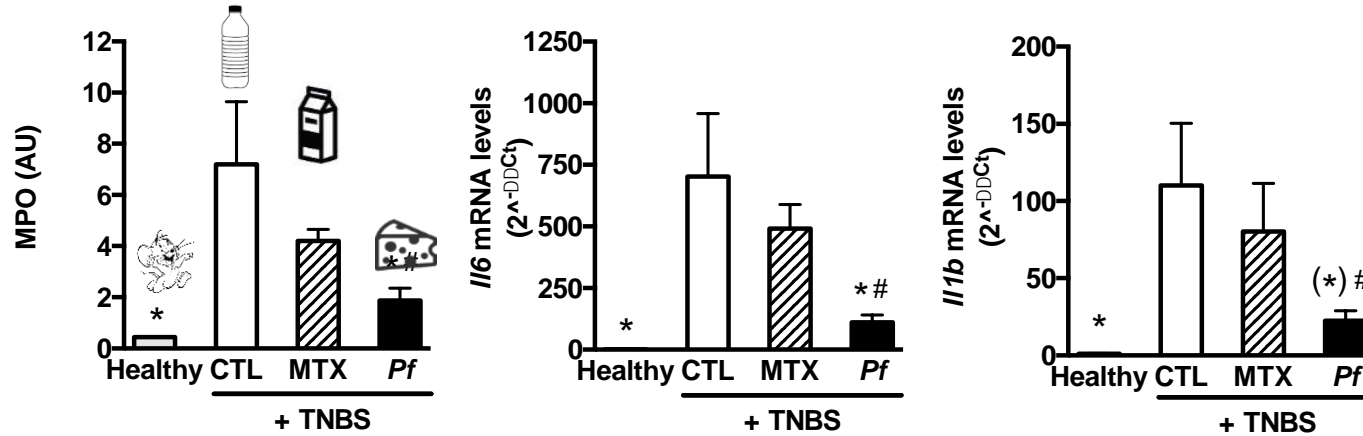
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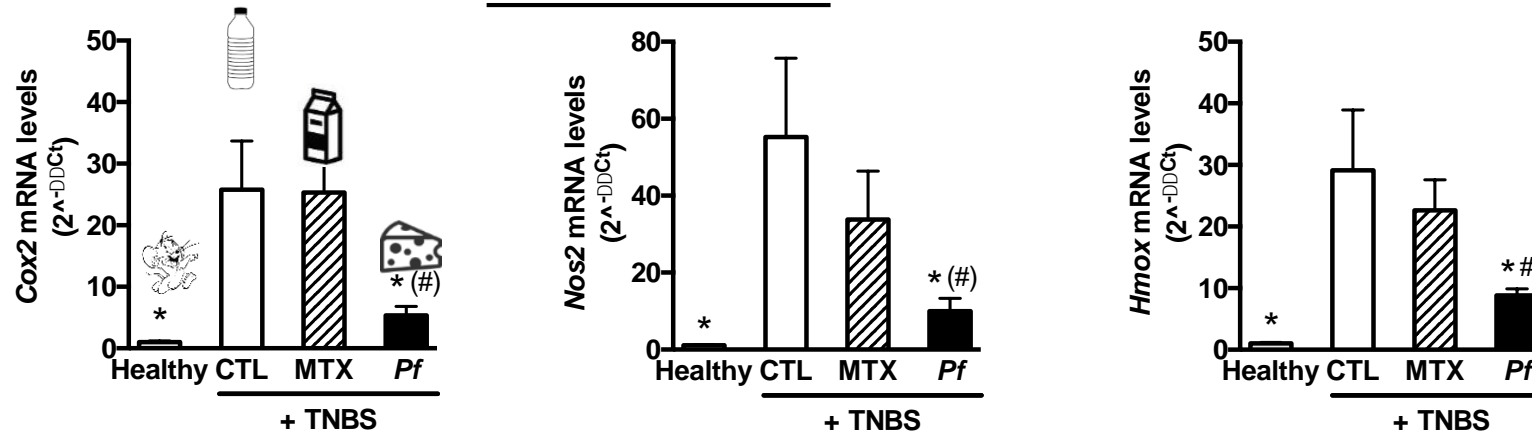


➤ Cheese prevents TNBS-induced colitis in mice

Colonic inflammatory markers



Colonic oxidative stress markers



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



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➤ 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!)



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Propionibacteria immunomodulation
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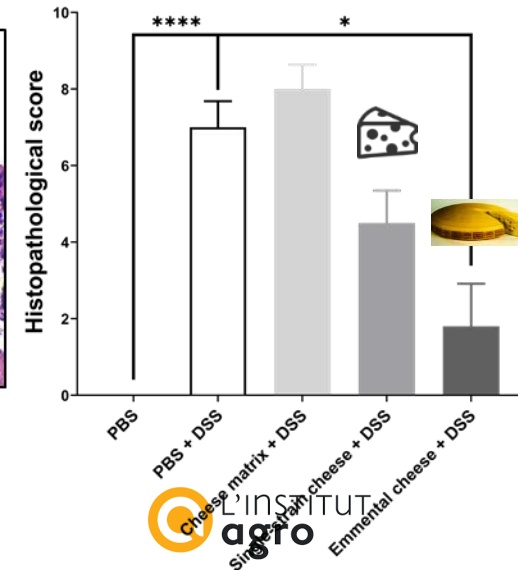
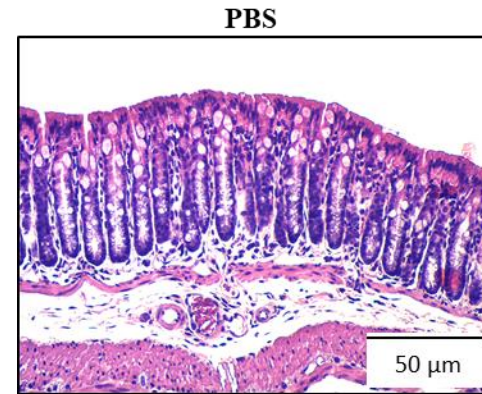
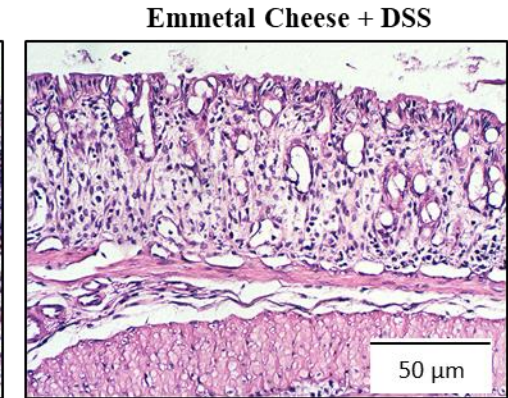
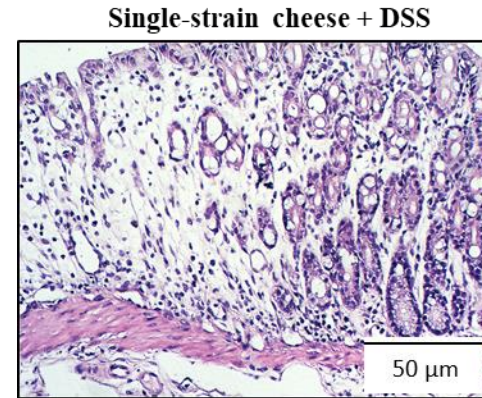
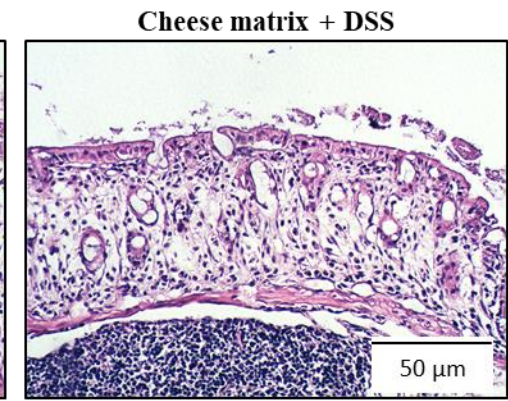
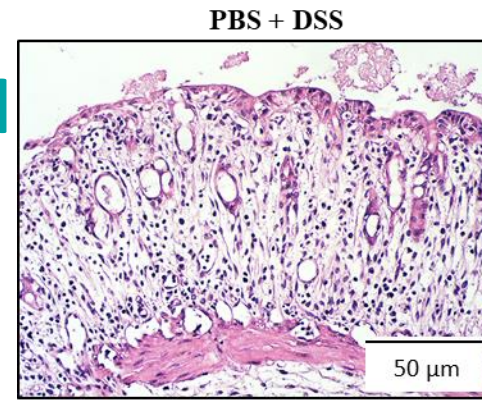
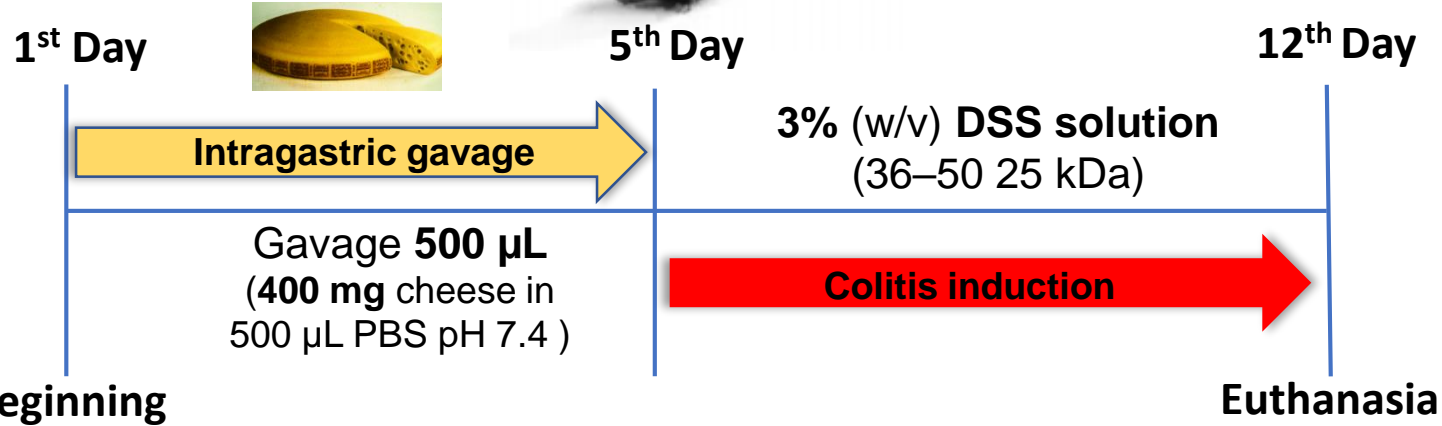


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➤ Emmetal: prevention of DSS-induced

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

C57BL6
8th week age



Vasco Azevedo



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Propionibacteria immunomodulation
Fat matters



STLO



L'INSTITUT
agro
SIN

➤ 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

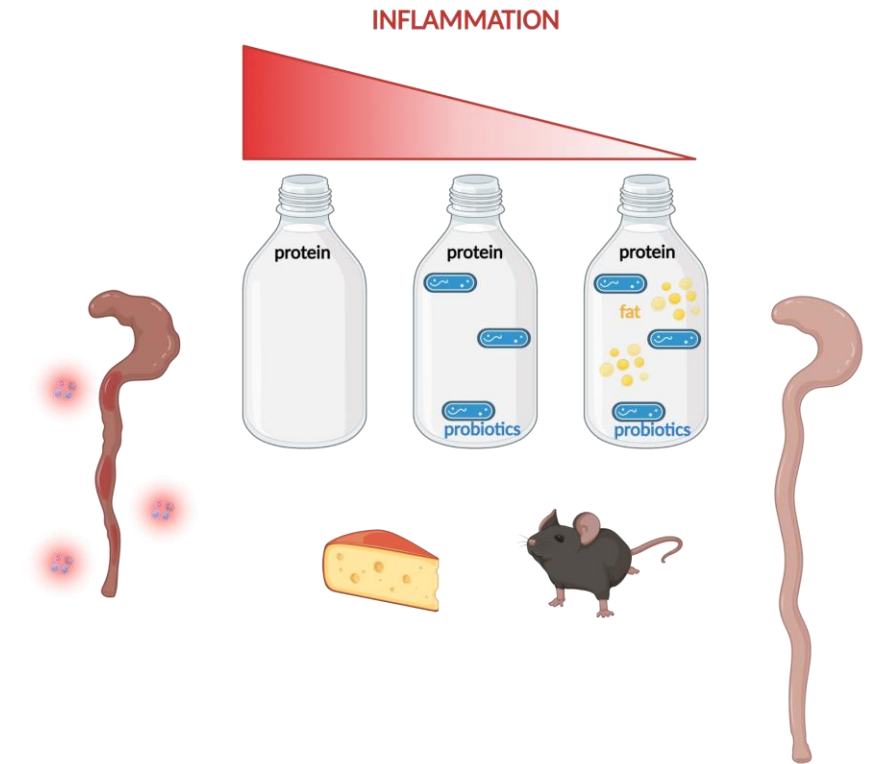
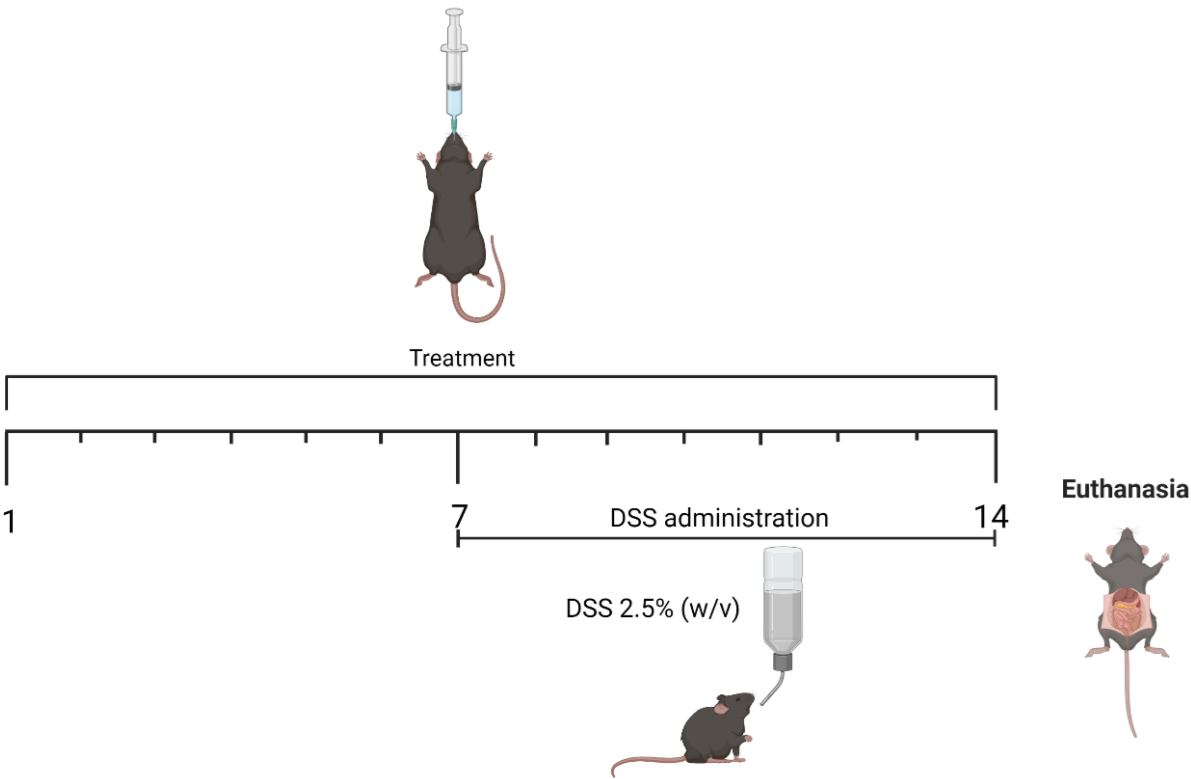


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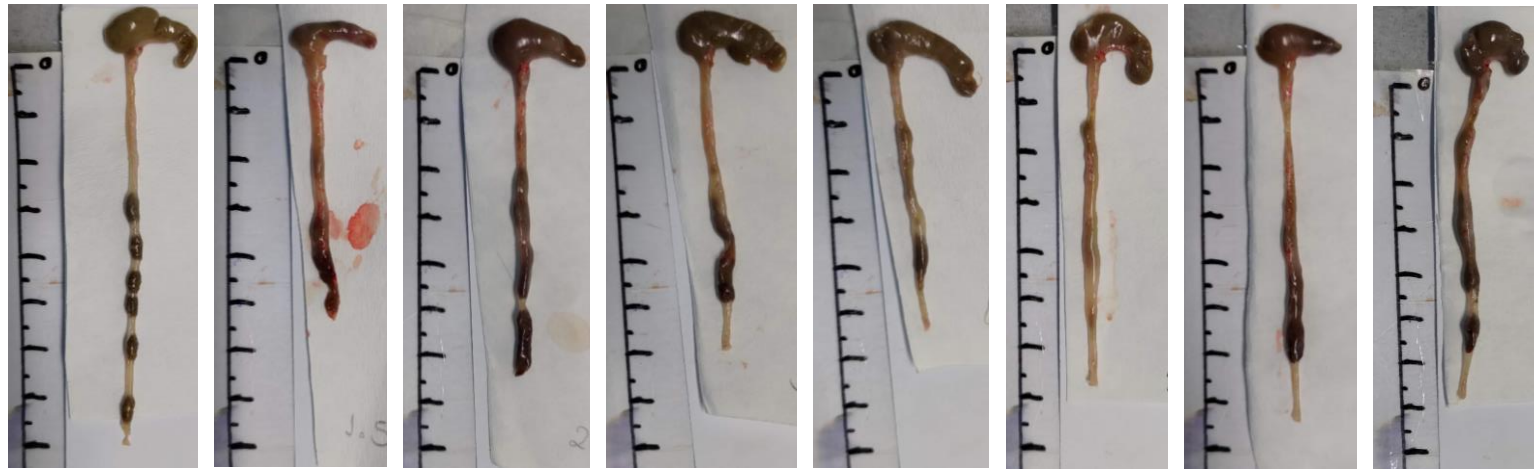
Propionibacteria immunomodulation
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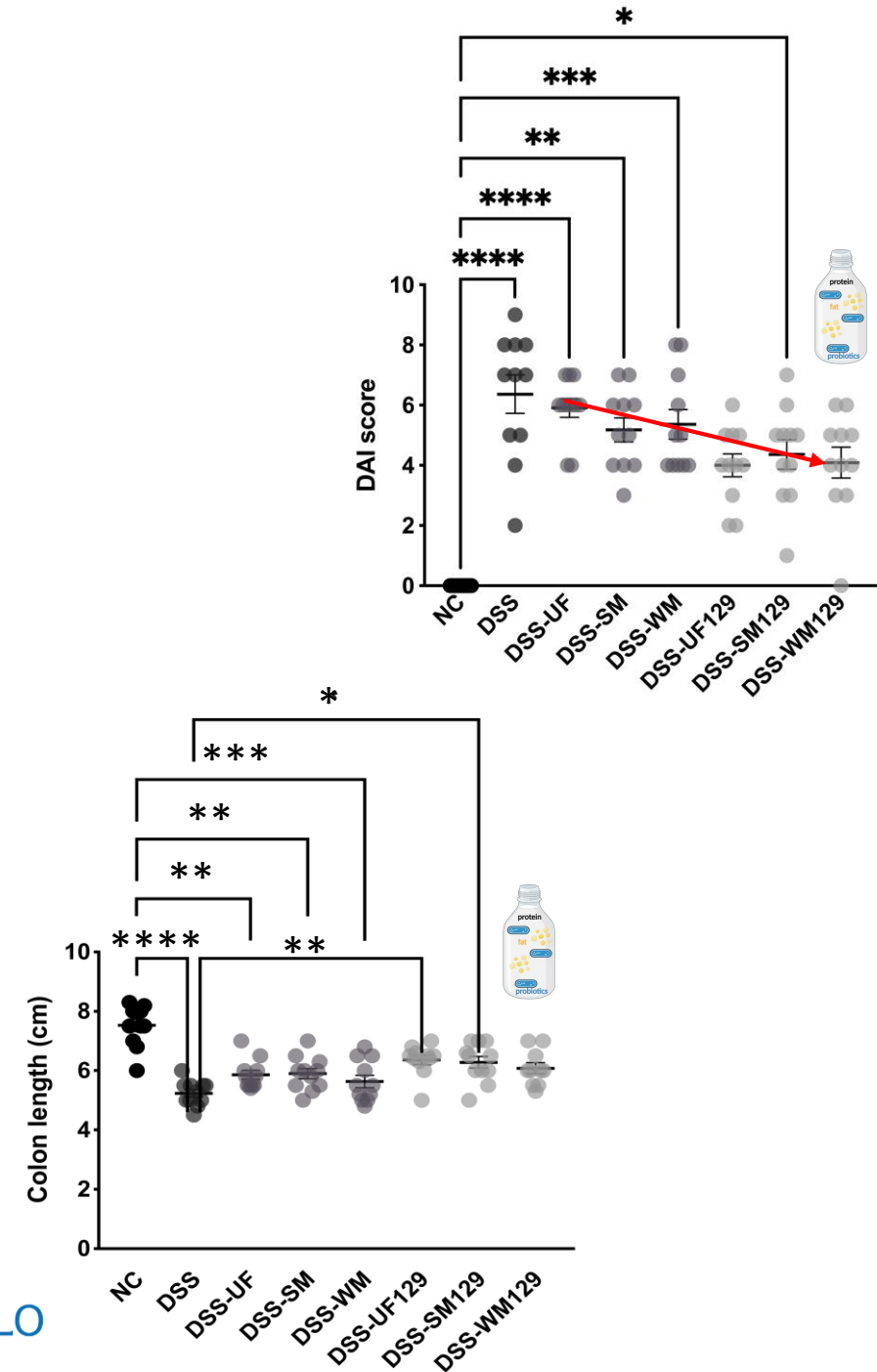
➤ Products given by gavage before colitis induction in mice



➤ Effect on colon and DAI



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



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Propionibacteria immunomodulation

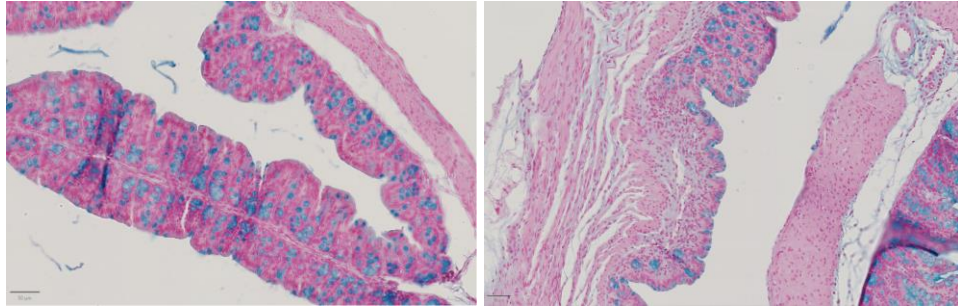
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UFMG
UNIVERSIDADE FEDERAL DE MINAS GERAIS

LGCM
Laboratório de Genética Celular e Molecular

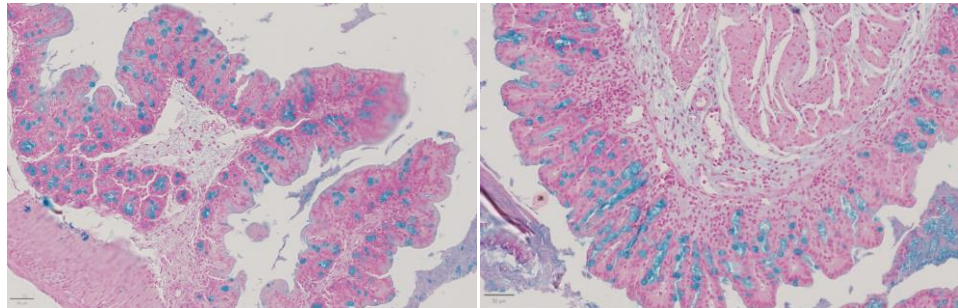
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➤ Only fermented whole milk protects the colon mucosa architecture



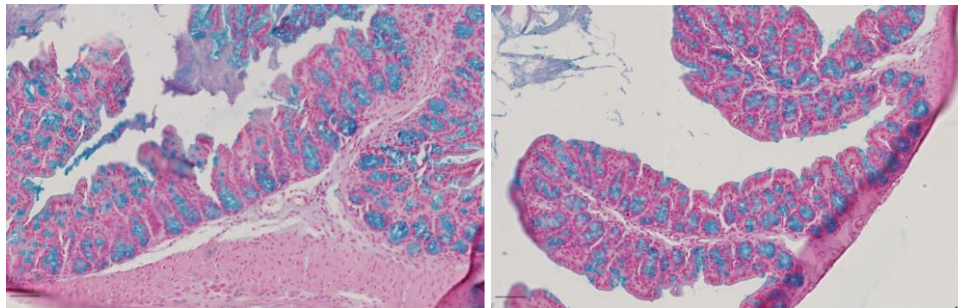
NC

DSS



DSS-WM

DSS-UF129



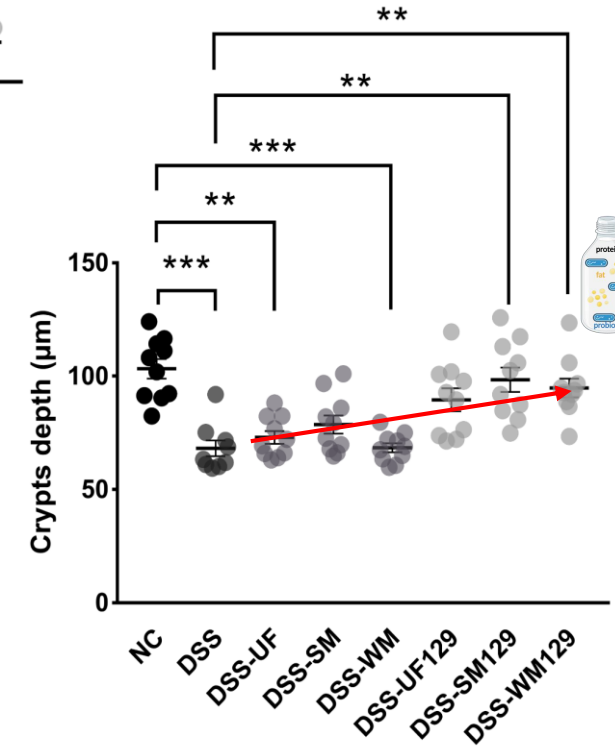
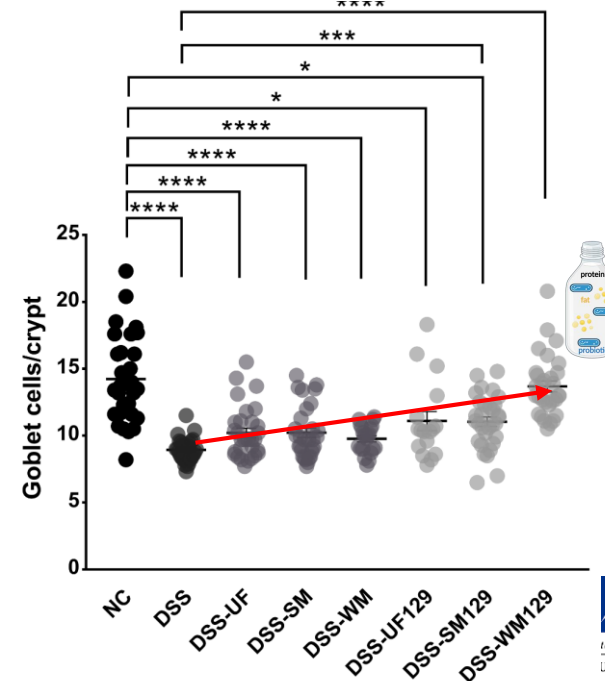
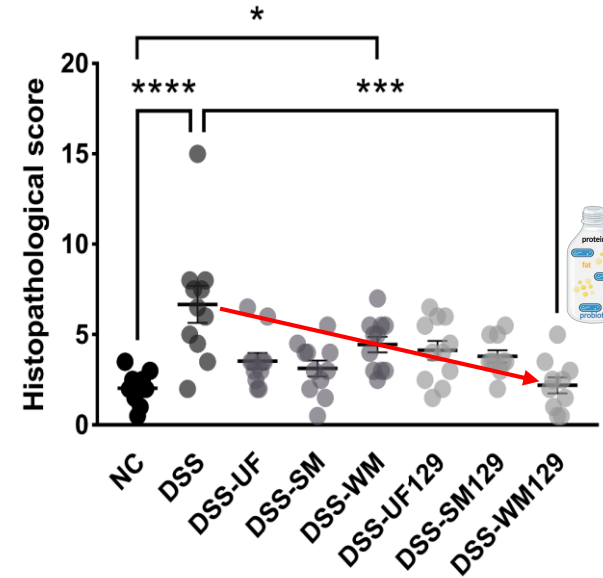
DSS-SM129

DSS-WM129

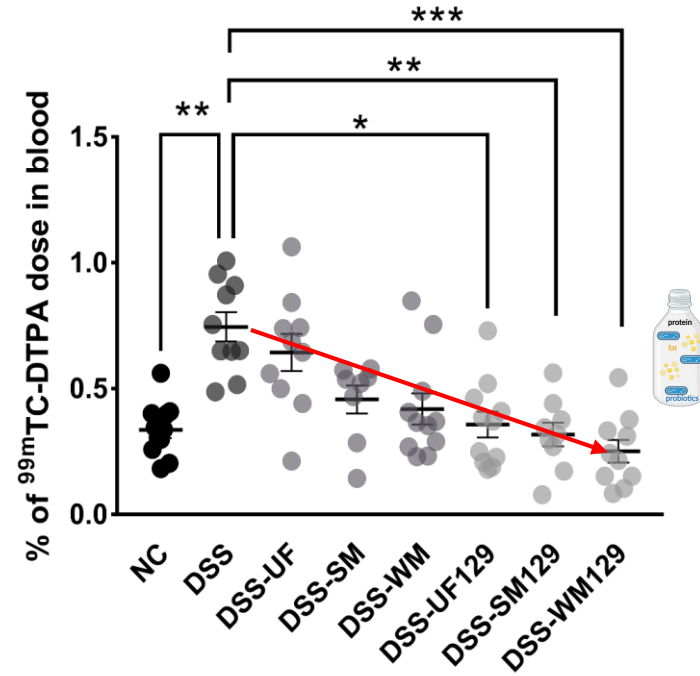
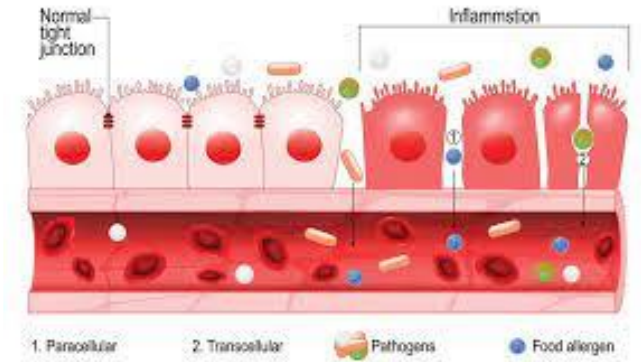
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Propionibacteria immunomodulation

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➤ Gut barrier permeability

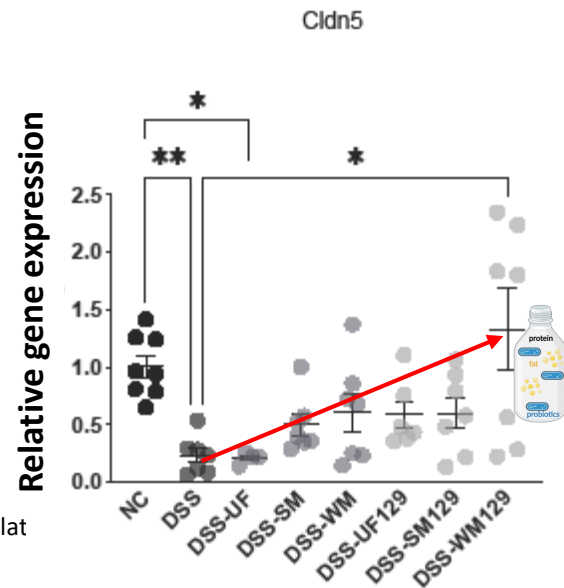
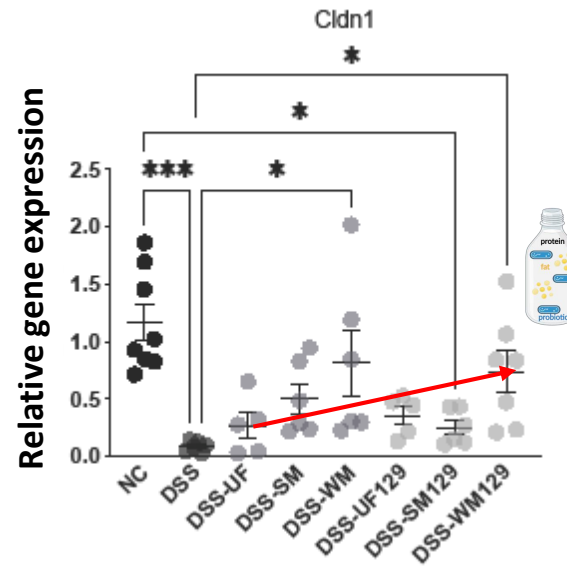
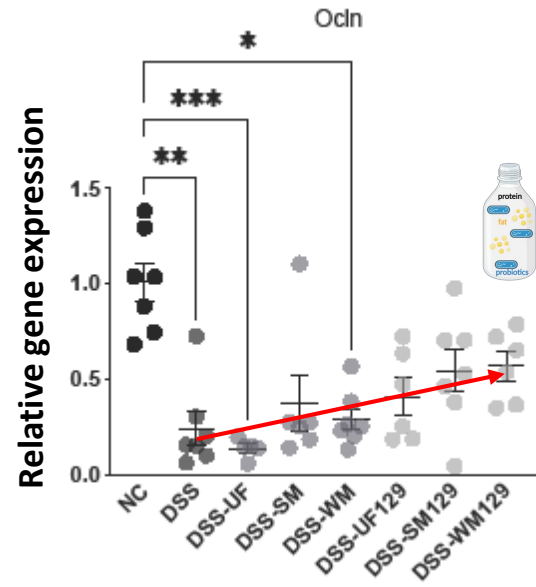


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➤ Gene expression



Relative gene expression

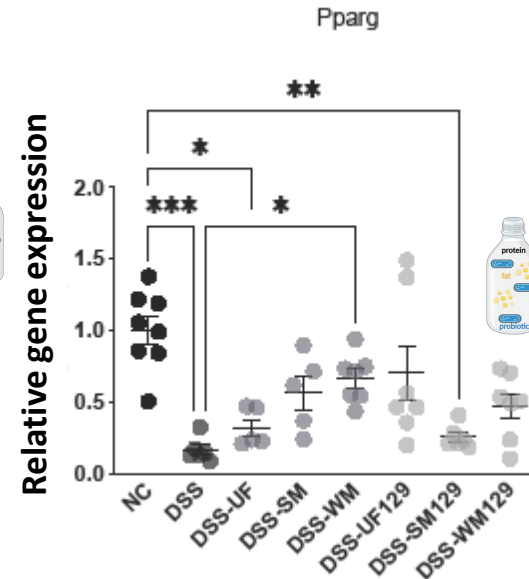
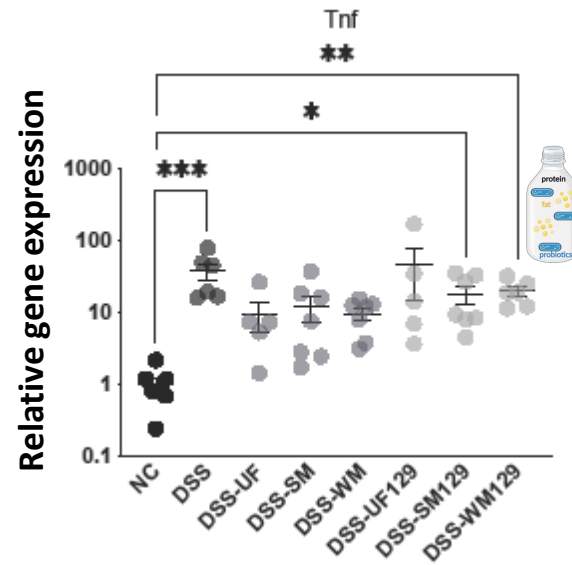
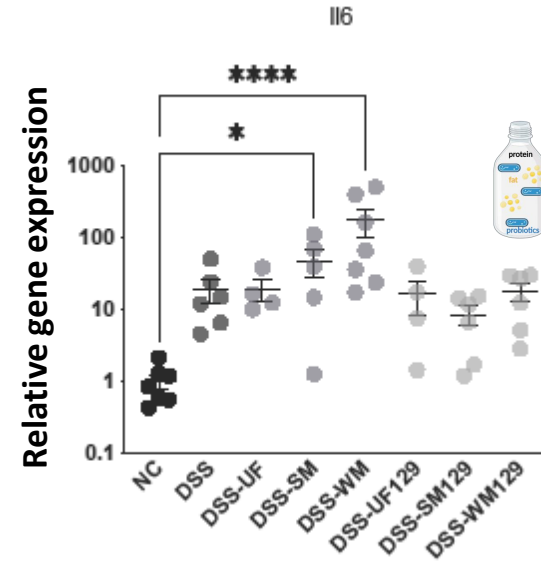
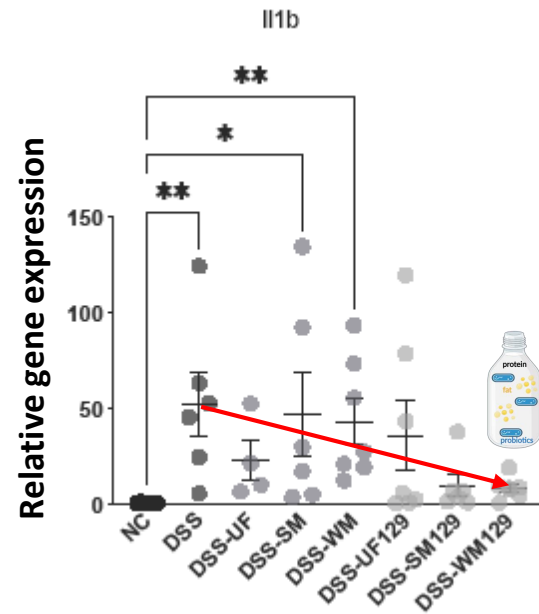


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➤ Gene expression



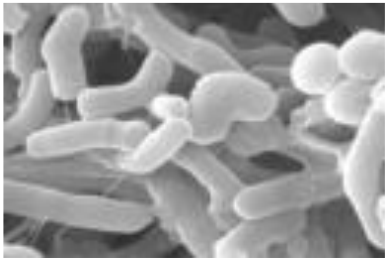
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Propionibacteria immunomodulation
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- Propionibacteria immunomodulatory probiotics
 - Fermented dairy products, good probiotic delivery vehicle
 - Dairy fat plays a role

➤ Thank you for your attention!
Any kind question?



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