A proteomic analysis of immunomodulatory effects mediated by the probiotic propionibacterium freudenreichii and by its EVs

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A proteomic analysis of immunomodulatory effects mediated by the probiotic *Propionibacterium freudenreichii* and by its EVs

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

Science & Technology of Milk and Eggs
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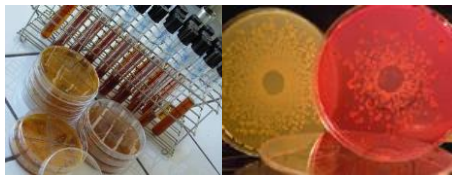
A multidisciplinary and multiscale approach, reinforced by two high-calibre facilities:

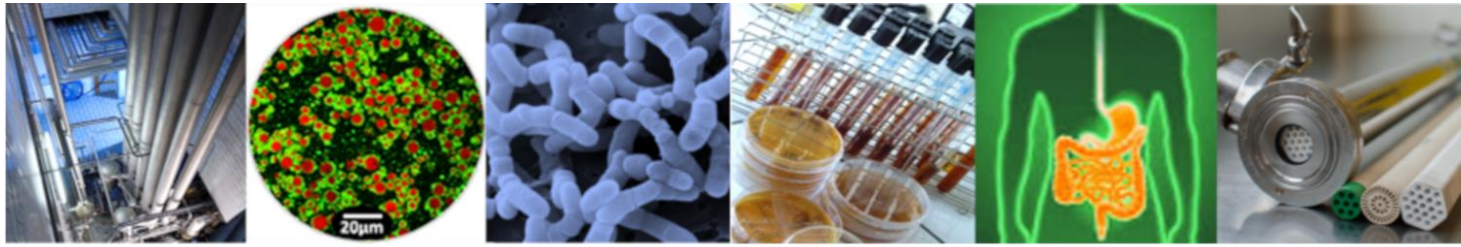
Dairy Platform

Biological Resource Centre

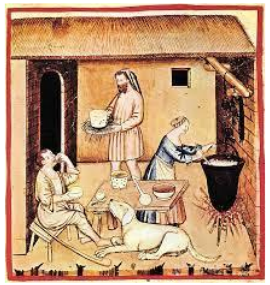


- **Structuration / destructuration mechanisms of food matrix:**
  from structural characterisation to digestion
- **Dairy processing and cheese making:**
  toward sustainable dairy systems
- **Microbial interaction:**
  food matrix and host cell

Propionibacterium freudenreichii: Who's that bug?



Emmental and other cheeses with holes: a long story



Flavour:

Fruity

Sour

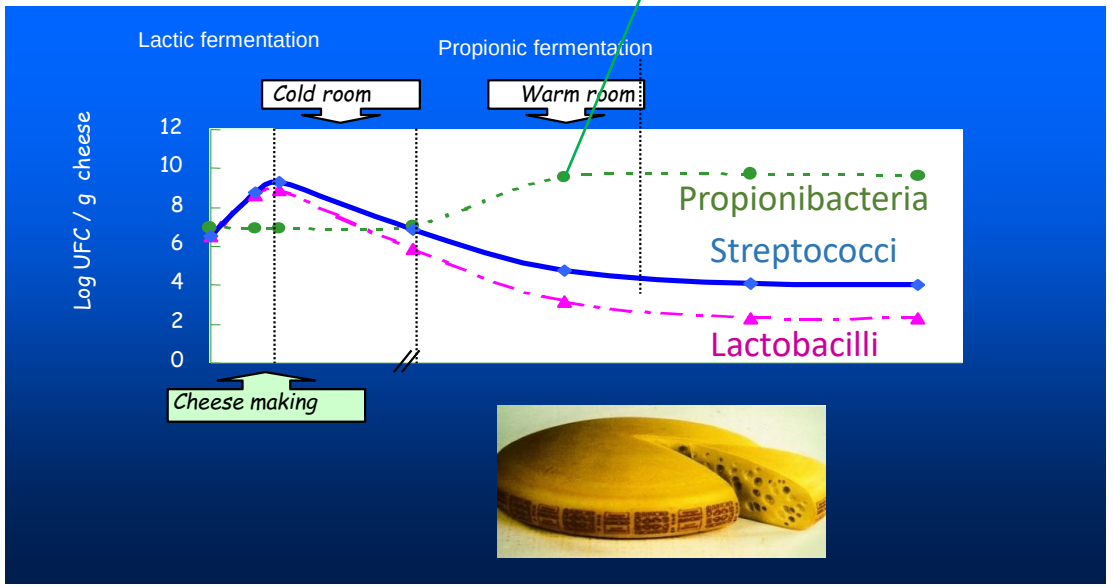
Picante

Propionate

Acetate

CO_2

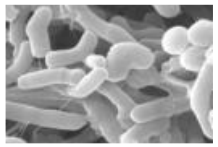
…Esters



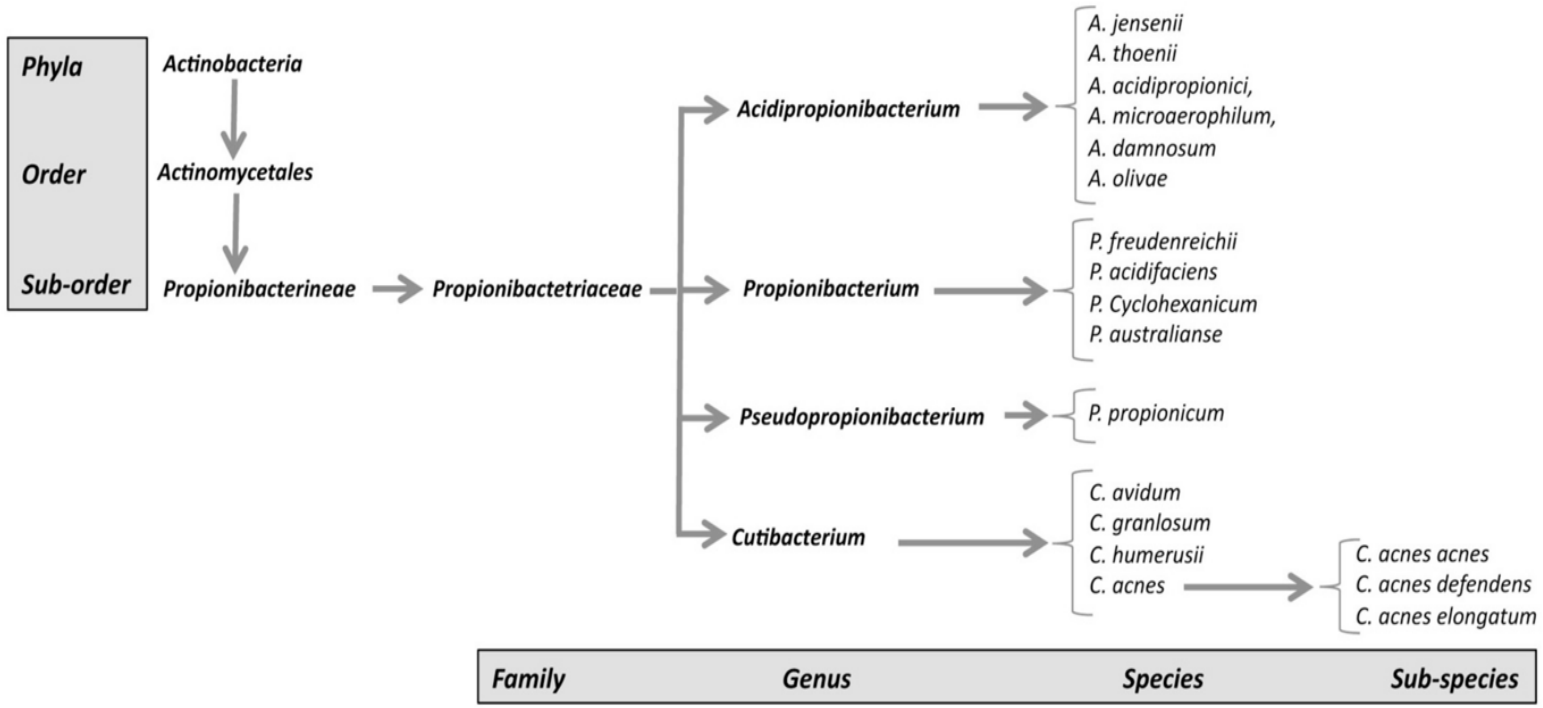
➔ **The Fitz equation (1878)**

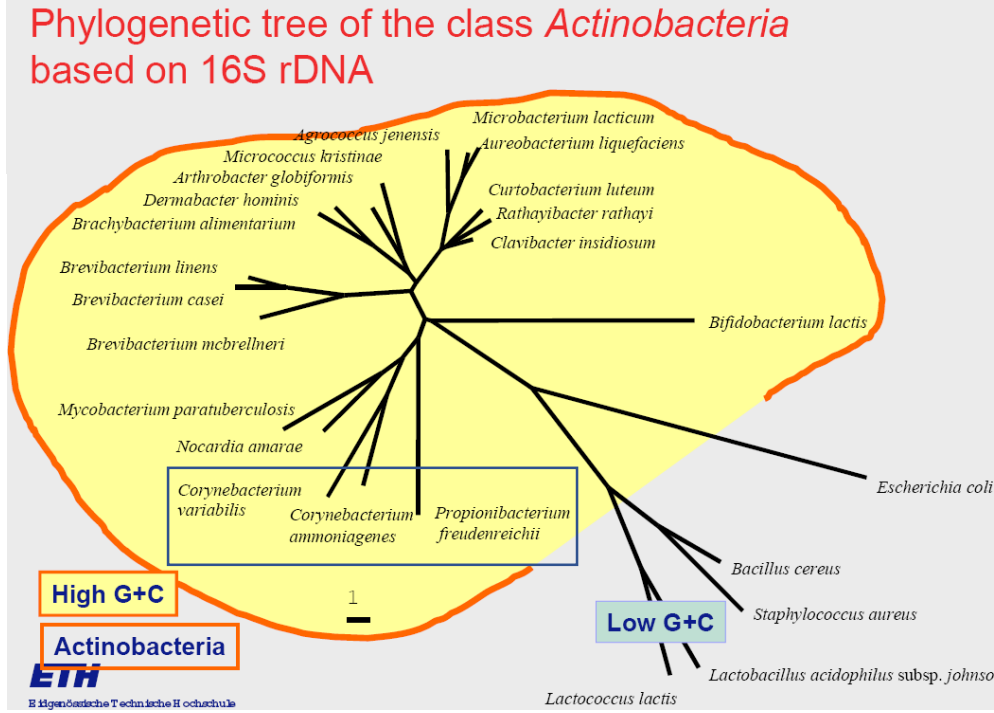
3 Lactate ⟶ 2 propionate + 1 acetate + 1CO_2

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



Propionibacteria, in a few words



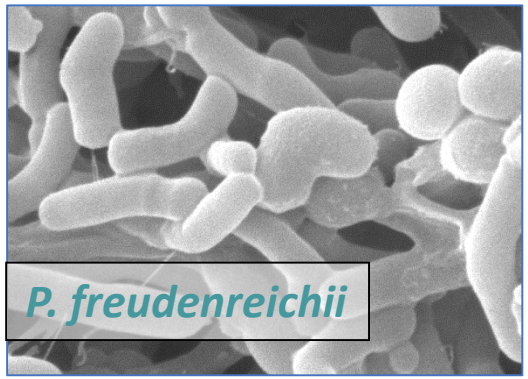


Gram +

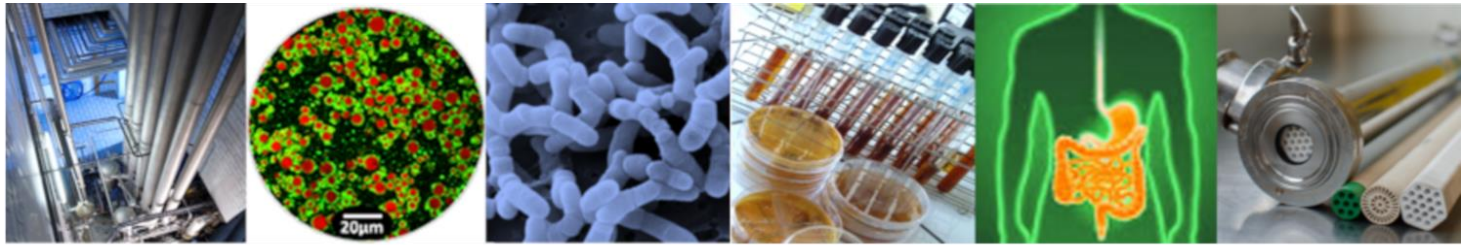
pleiomorphic

anaerobic to microaérophilic

non motiles et non sporulated



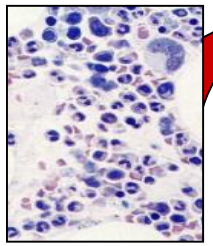
Propionibacterium freudenreichii: An inducer of anti-inflammatory cytokines



Cytokine stimulation on human PBMCs (mononucleated leucocytes from donors)

Bruno Pot

Benoit Foligné



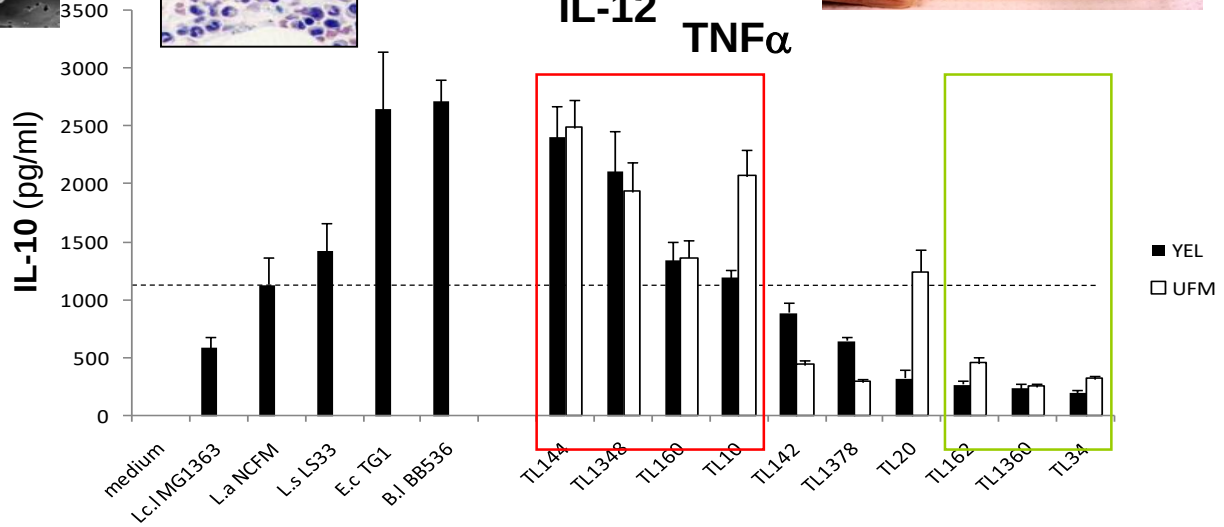
24 h

IL-10

IFNγ

IL-12

TNFα

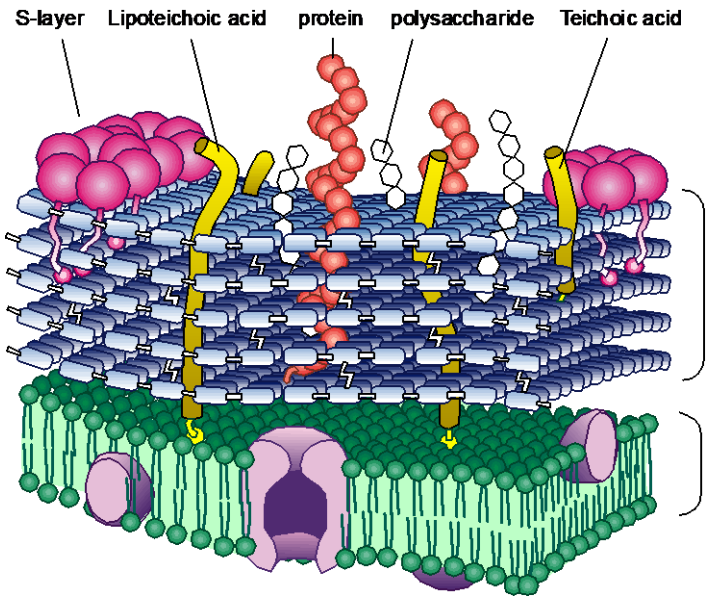


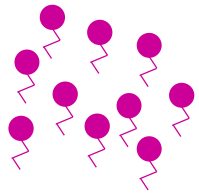
The best anti-inflammatory strains

The less anti-inflammatory strains

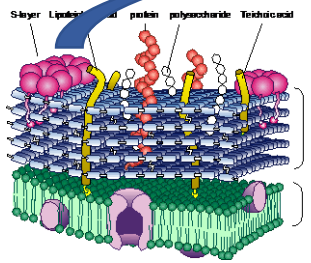
Cytokine stimulation: surface layer proteins (SLP) involved

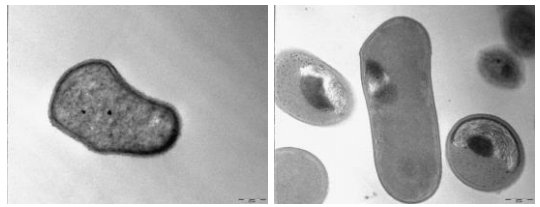
Guanidine Chloride





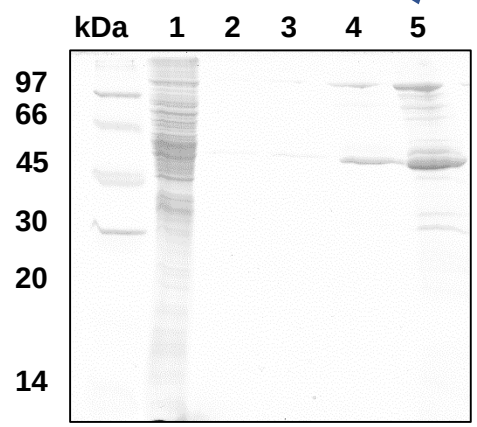
A key role of surface proteins

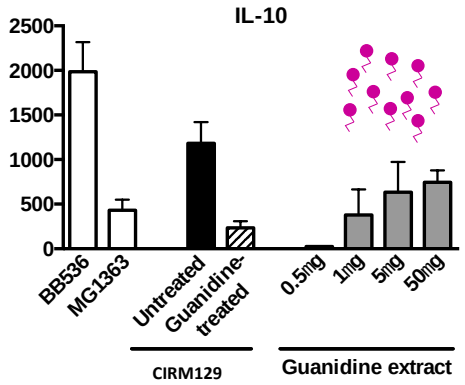




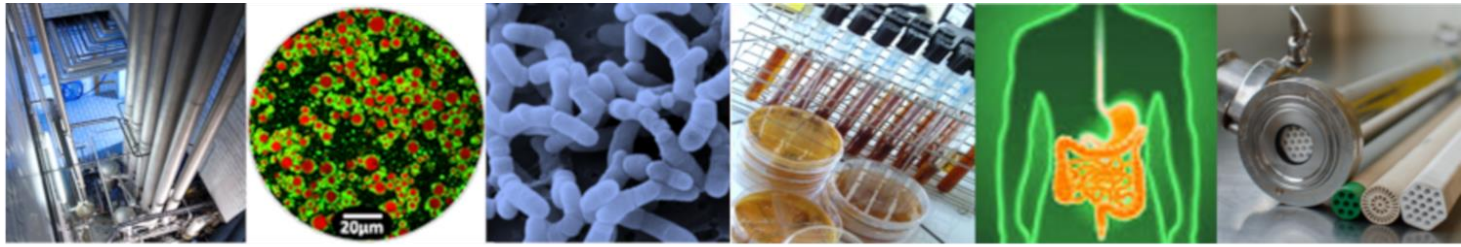
Untreated Guanidine treated

Guanidine Chloride

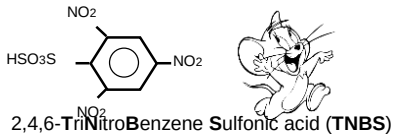




Propionibacterium freudenreichii: Anti-colitis effects demonstrated *in vivo*

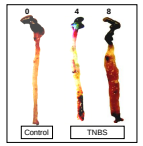


Propionibacteria: prevention of TNBS-induced colitis in mice

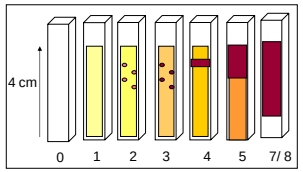


2,4,6-**Tri**Nitro**B**enzene **S**ulfonic acid (**TNBS**)


Acute colitis

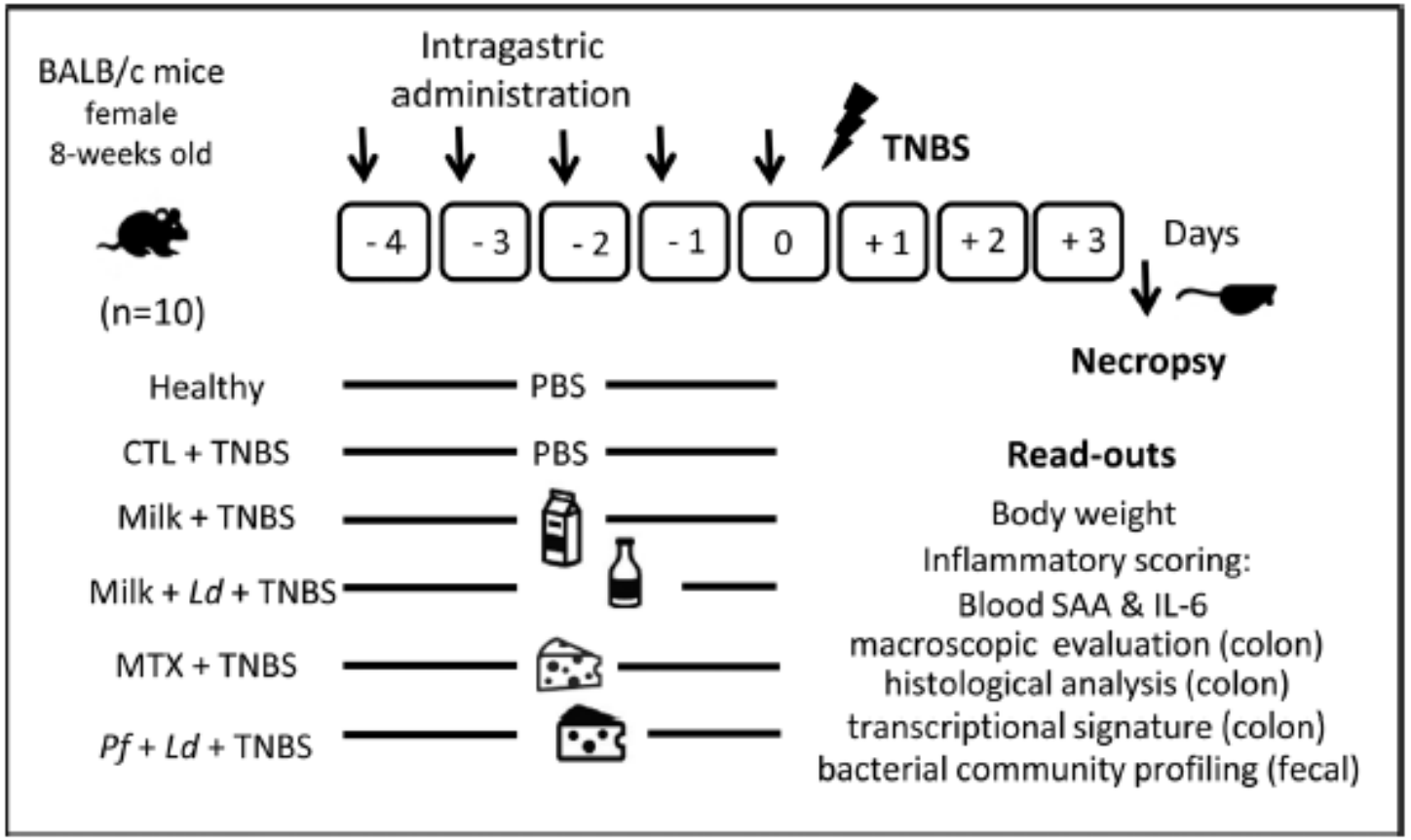


Macroscopic score (Wallace)

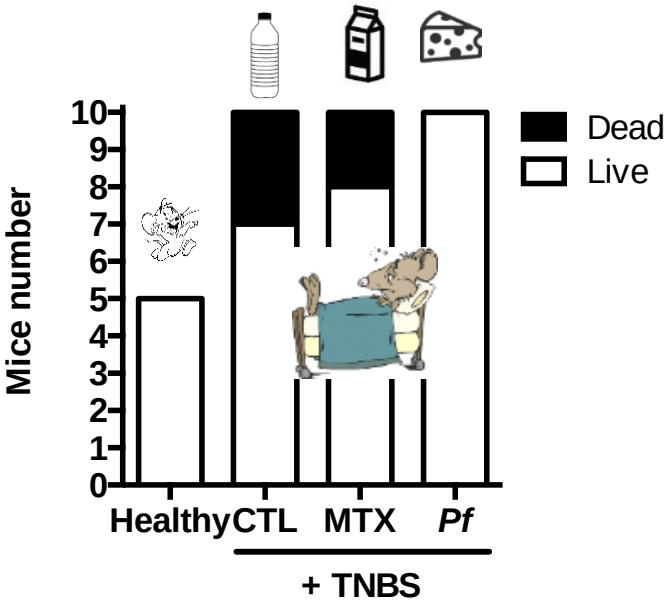



Bruno Pot

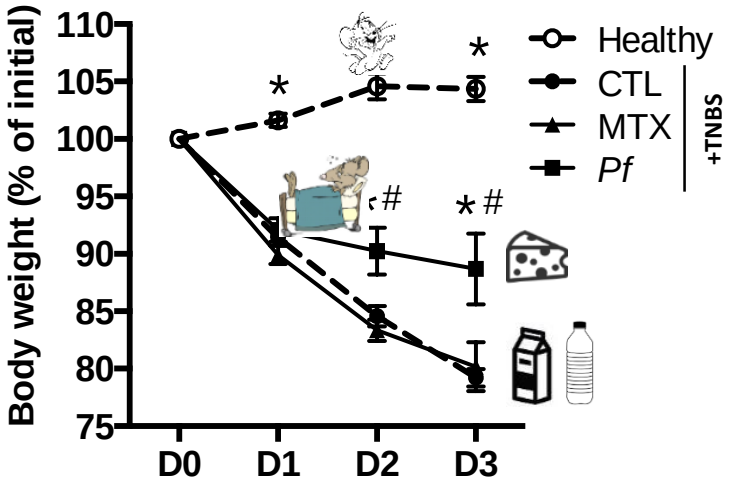

Benoit Foligné



Propionibacteria: prevention of TNBS-induced colitis in mice

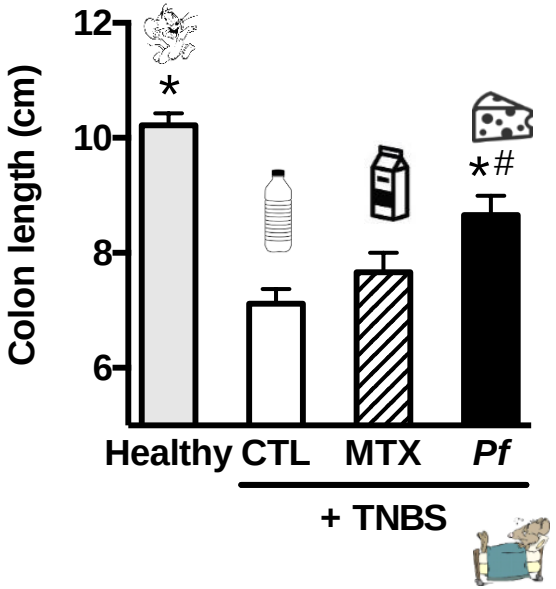


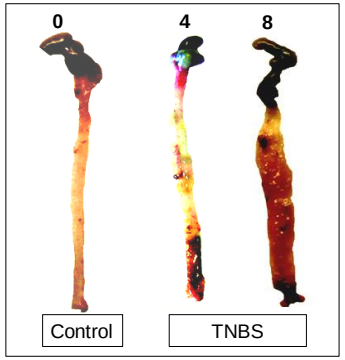
Survival



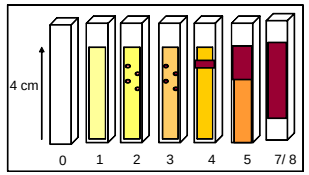
Body weight

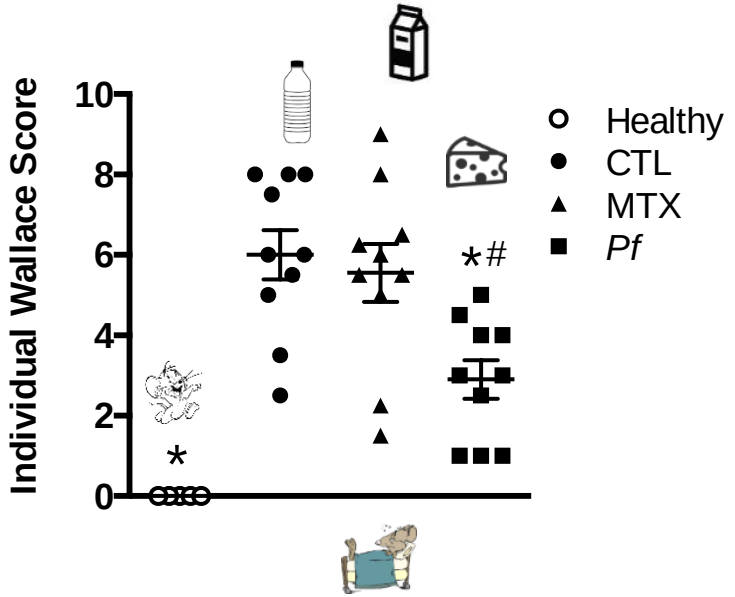
Propionibacteria: prevention of TNBS-induced colitis in mice



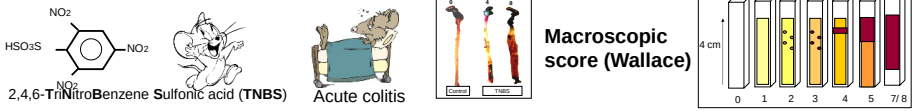


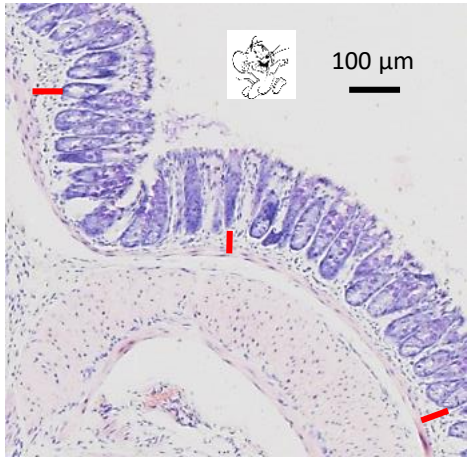
Macroscopic score (Wallace)

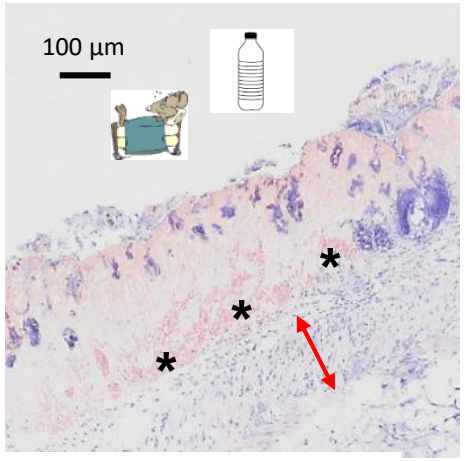


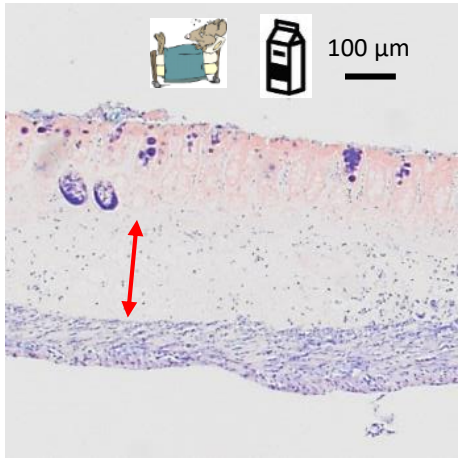


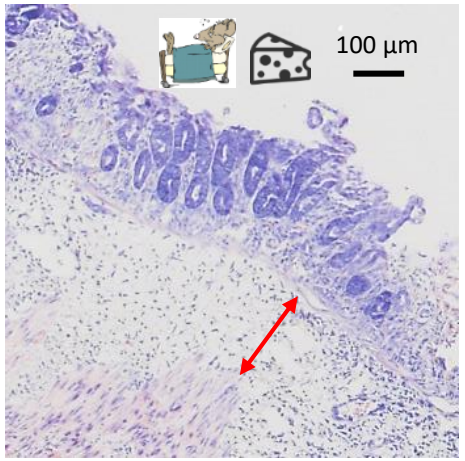
Propionibacteria: prevention of TNBS-induced colitis in mice

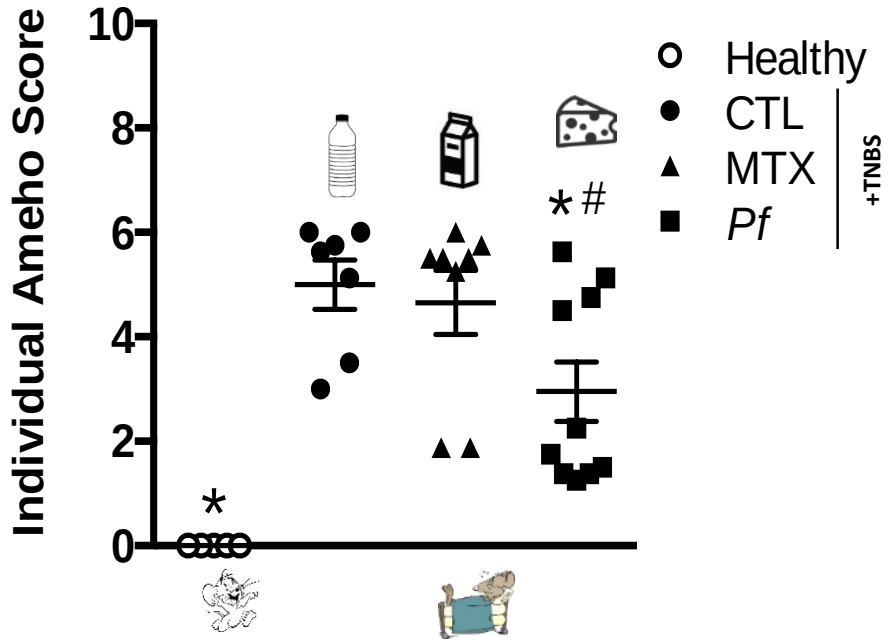






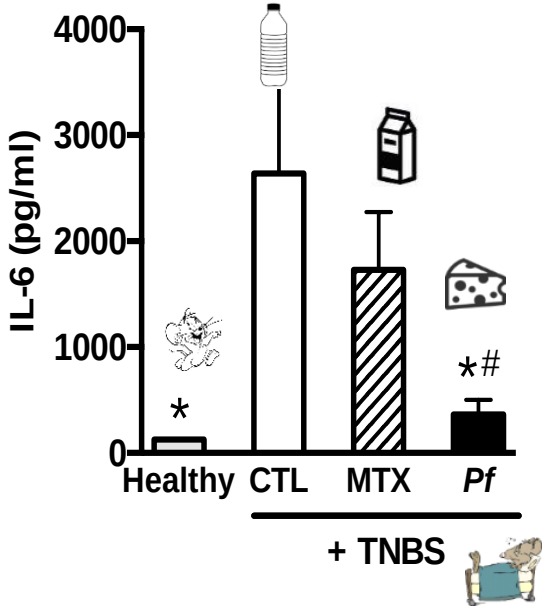




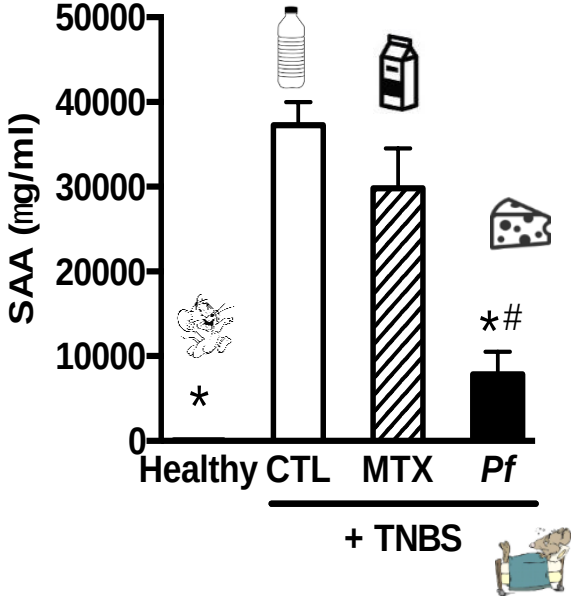


Propionibacteria: prevention of TNBS-induced colitis in mice

Blood inflammation markers



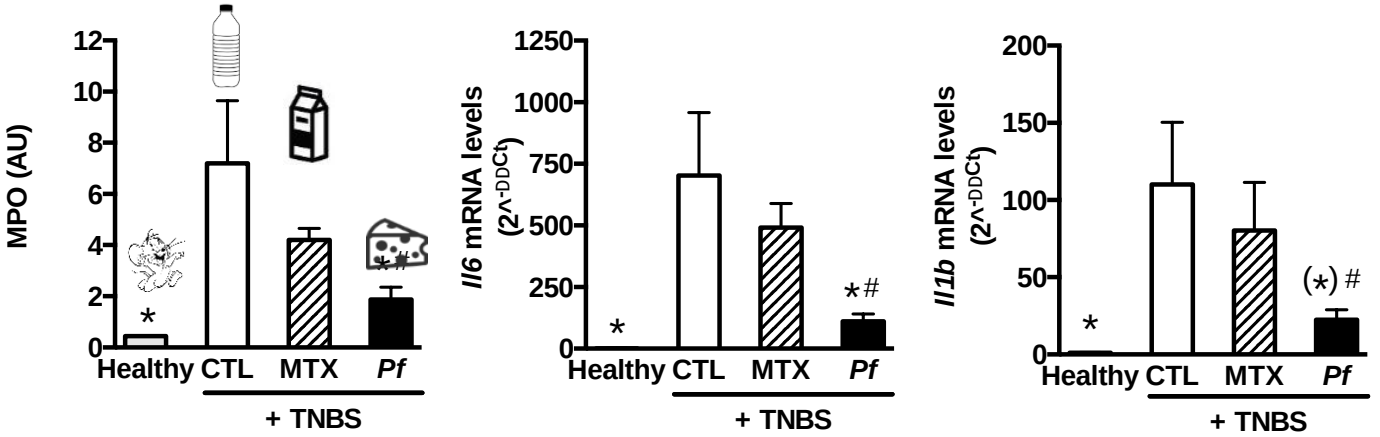
Interleukin 6

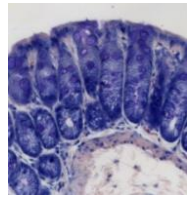


Serum Amyloid A

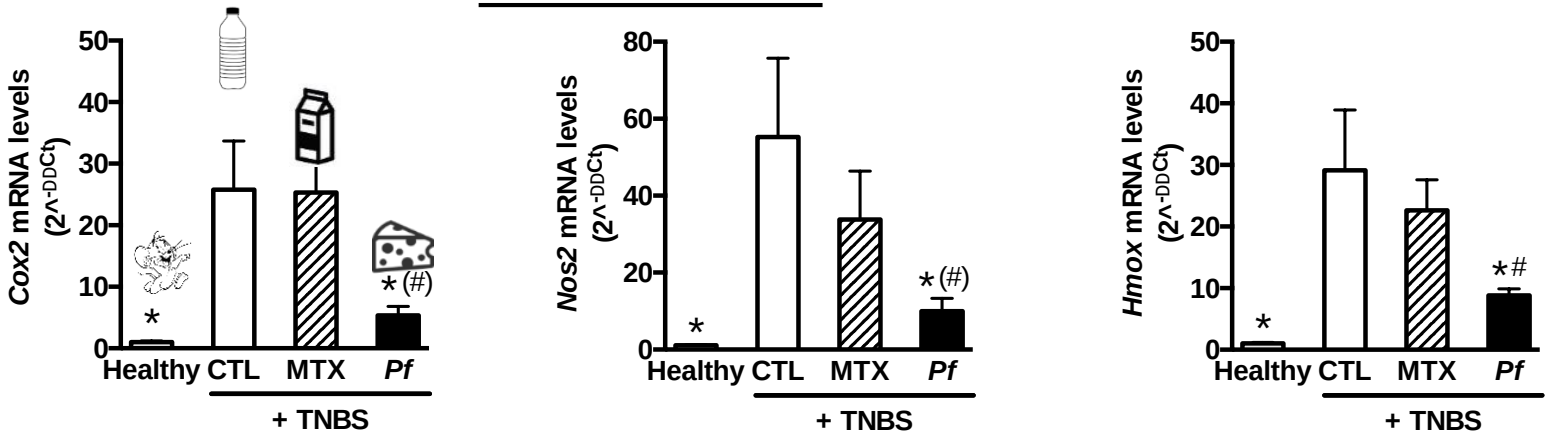
Propionibacteria: prevention of TNBS-induced colitis in mice

Colonic inflammatory markers



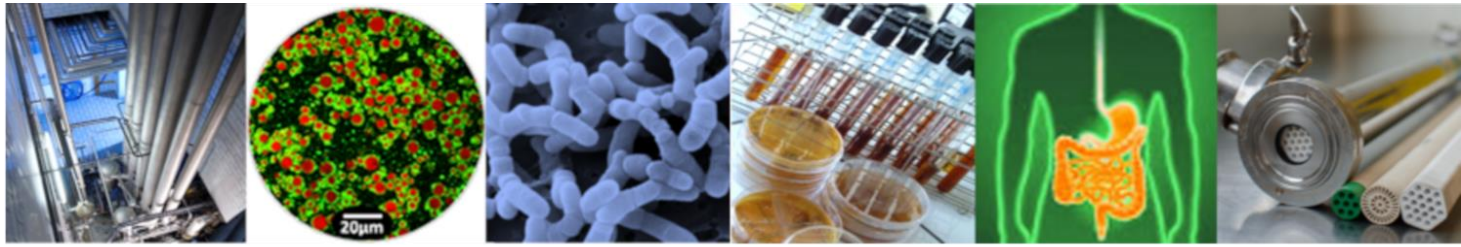


Colonic oxidative stress markers

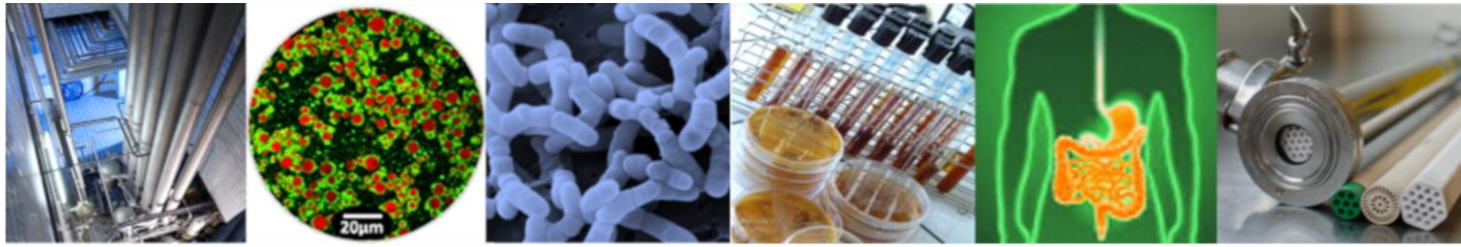


* p<0.05: vs CTL
\# p<0.05: vs MTX

How does it work?



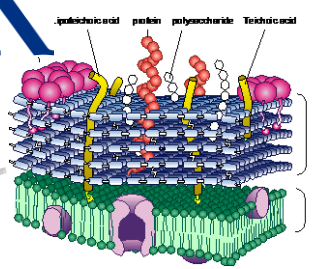
Comparative proteomics related to IL-10 induction



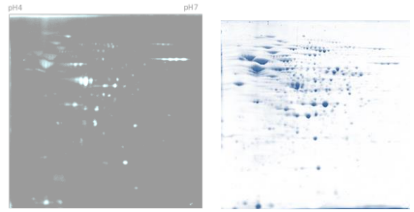
Looking for correlations

SURFING
Starter Surface against Inflammation of the Gut

Agence Nationale de la Recherche
ANR



Surfaceome



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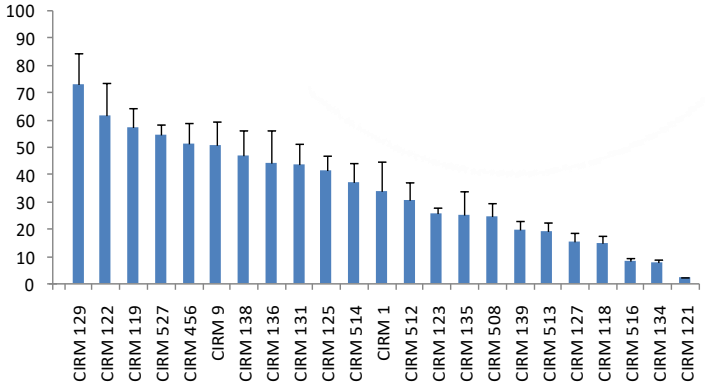
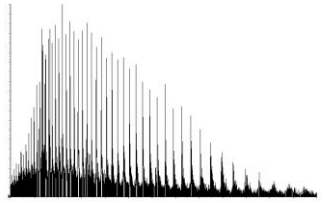


***In vitro* : IL-10**



julien.jardin@rennes.inra.fr

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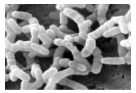
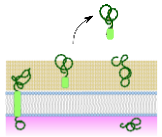
The surface proteomic approach

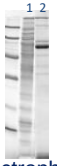


Bacteria grown in a fermented dairy product



Surface proteins guanidine extraction

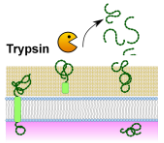
Dialysis



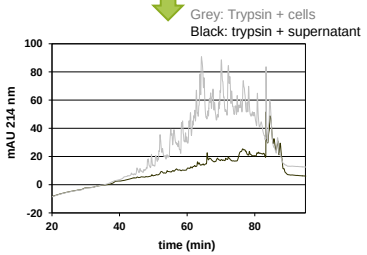
1D Electrophoresis

1: whole cell SDS extract ; 2: Guanidium extract

Band picking & trypsinolysis

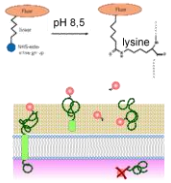


Surface proteins trypsin Shaving

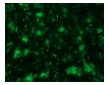


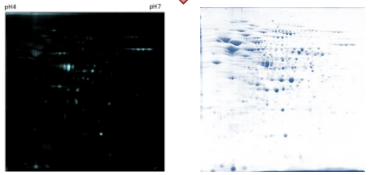
Released peptides

Mass spectrometry: NanoLC-ESI-MS/MS on ABSciex Q-Star XL



Surface proteins CyDye fluorescent labelling

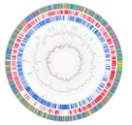




2D Electrophoresis

Spot picking & trypsinolysis

Database of predicted proteins



P. freudenreichii **genome sequence**

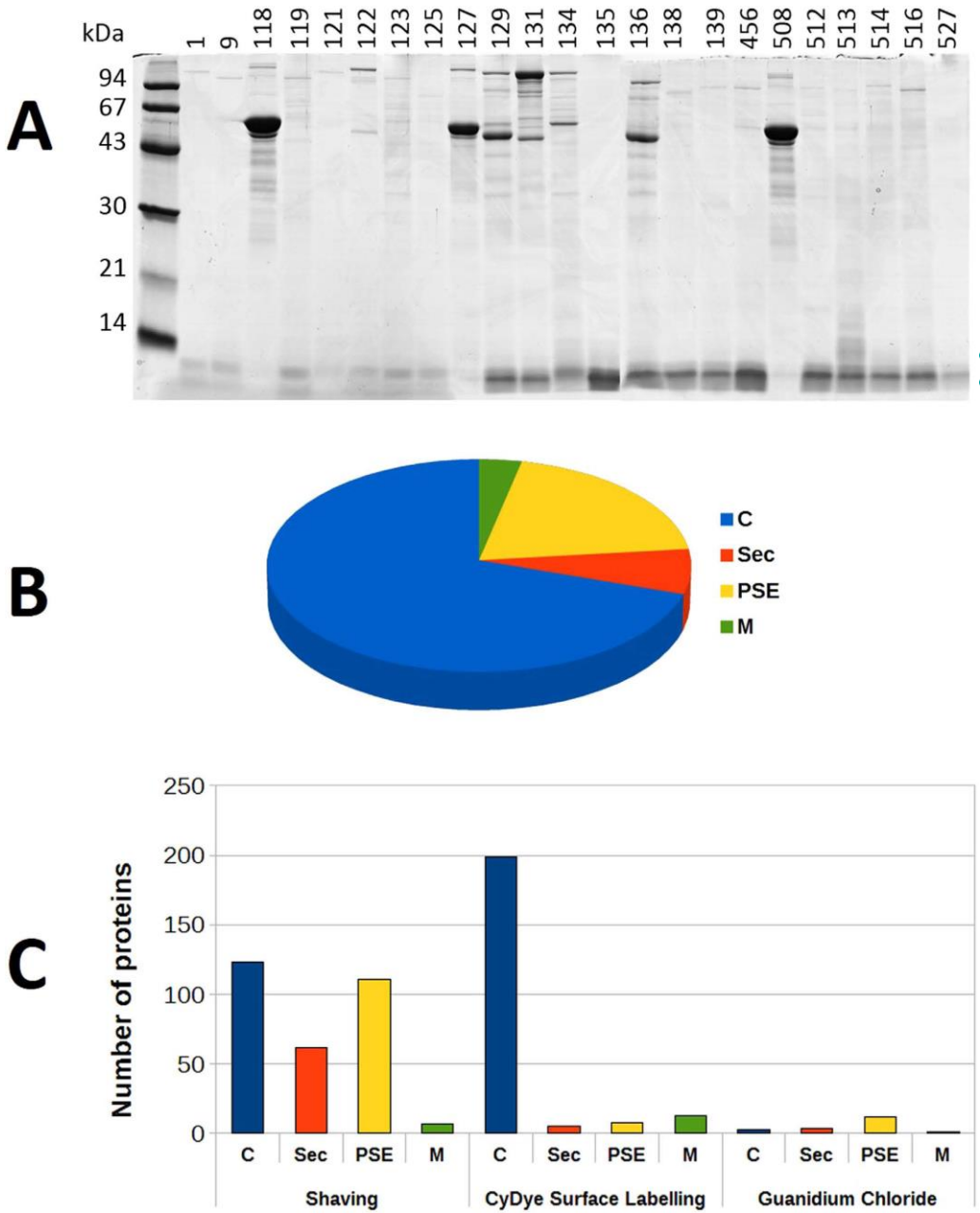
AGMIAL

Annotation on the Agmial platform

http://genome.jouy.inra.fr/agmial/

Localization prediction using SurfG+

Proteomics 9:61-73 (2009)

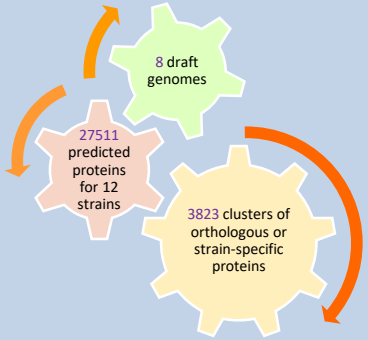


Diversity of *Propionibacterium freudenreichii* surface proteome, distribution into *in silico* predicted localisations.

(**A**) Guanidine hydrochloride-extracted proteins, analysed by SDS PAGE, evidenced variability among the *Pf* strains. (**B**,**C**). An inventory of the surface proteins of 12 *Pf* strains was further made by combining the three surface proteomic methods and produced a total of 509 proteomic identifications representing 174 different proteins. (**B**) Global distribution of *in silico* predicted localisations of the 174 different proteins identified. (**C**) Number and predicted localisations of the proteins identified using each analytical method: shaving, cyDye surface labelling and guanidine hydrochloride extraction (Le Maréchal *et al.*19). The localisations were predicted by SurfG + software, as described by Barinov *et al.*20 and the categories were as follows: (**C**) cytoplasmic protein, PSE: Protein surface exposed, M: membrane protein, SEC: secreted protein.

Treating correlations : statistics

Sequence & annotation
25 génomes

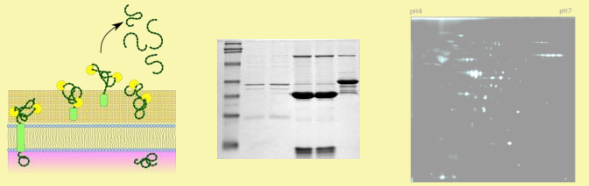


ARN

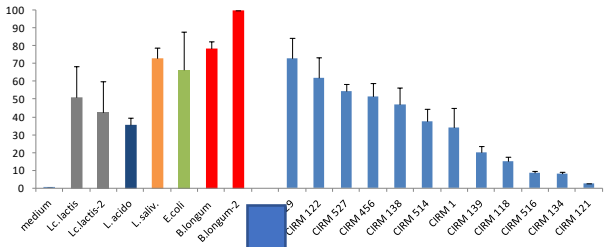
Transcriptomic: Nimblegen 72 K pangenomic microarray

Surface Proteins

3 proteomic methods



Phenotype (IL10)



R^2

R^2

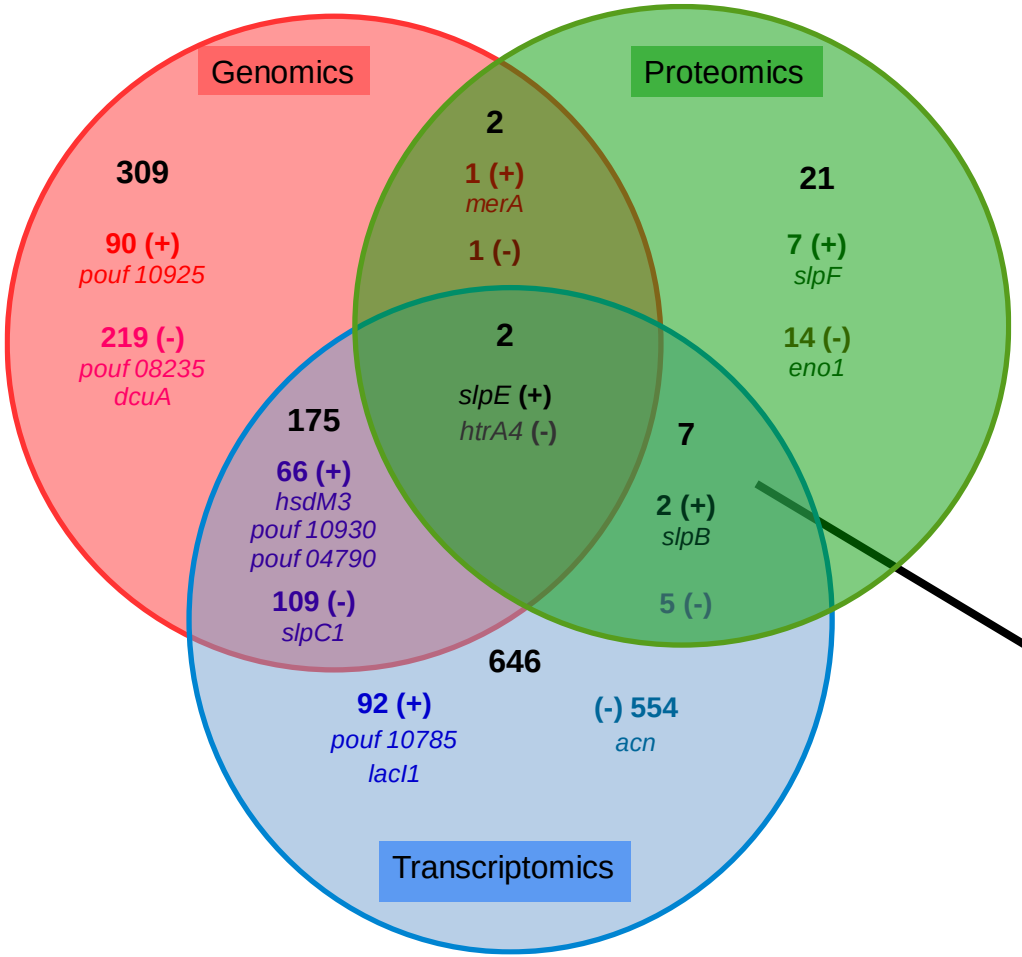
R^2

Candidate genes

Candidate RNAs

Candidate proteins

Treating correlations : statistics

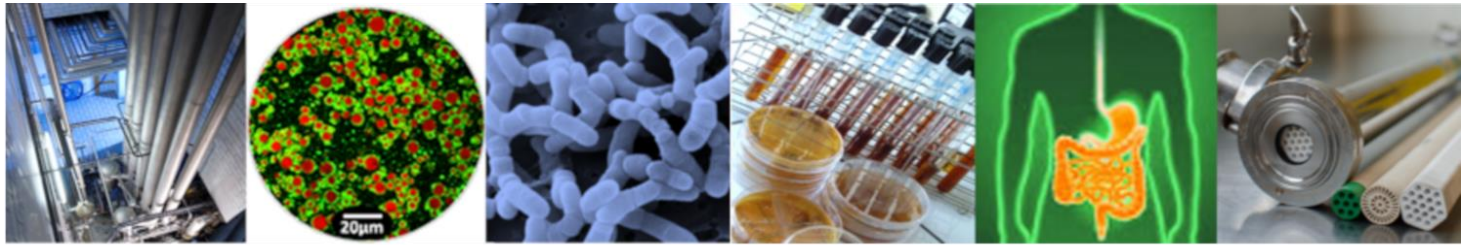


Mahendra.Mariadassou@jouy.inra.fr

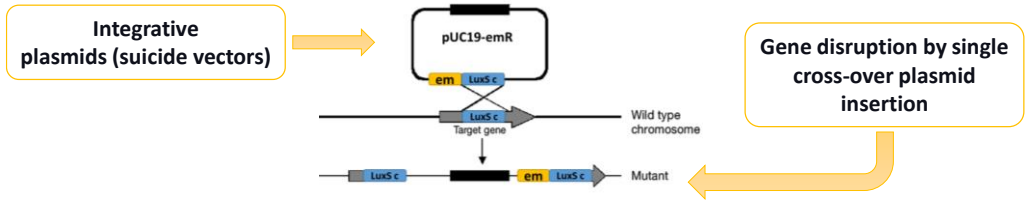
➢ Surface Layer Proteins

➢ A total of 45 genes inactivated

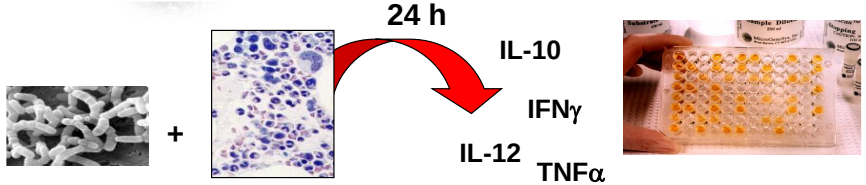
Mutational inactivation of surface layer protein genes

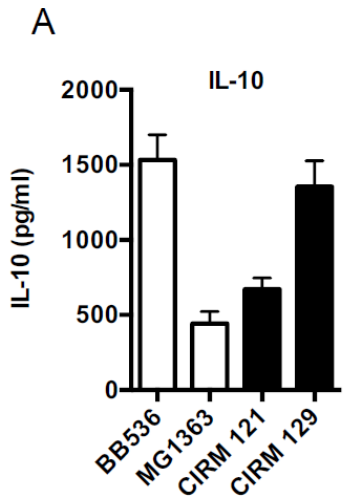


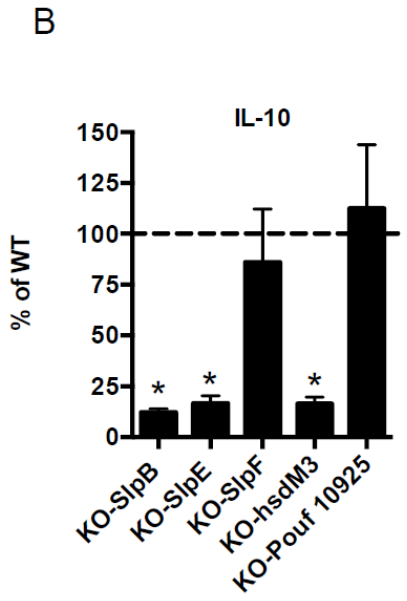
Impact on IL-10 induction



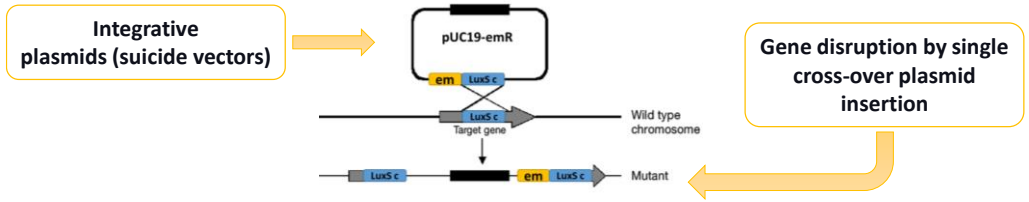
♦ *In vitro*

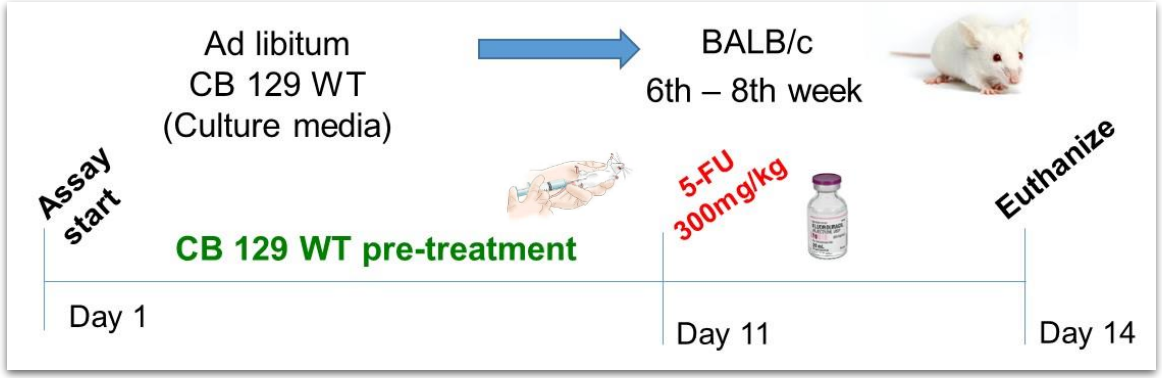


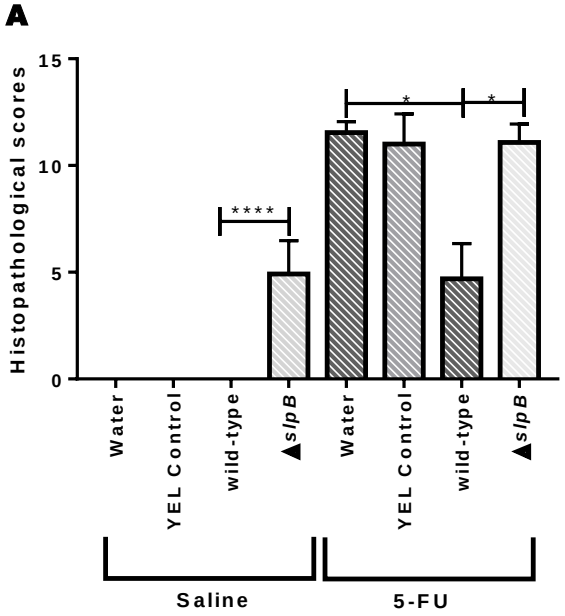


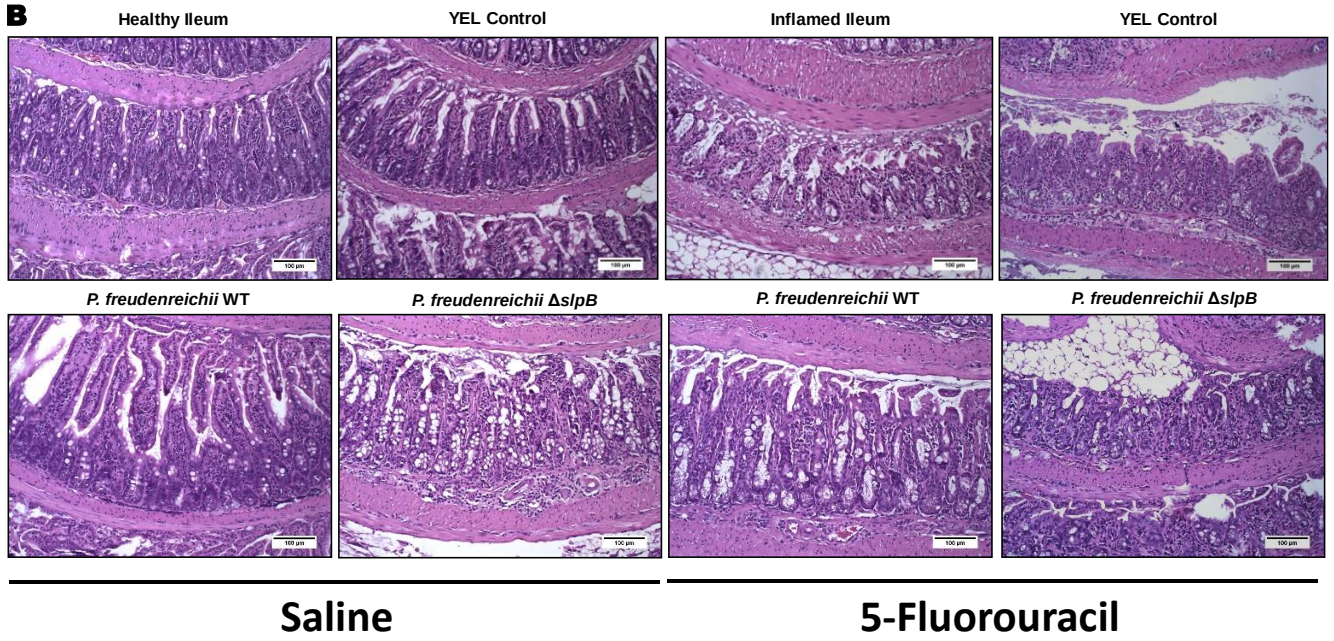


Impact on anti-inflammatory effect









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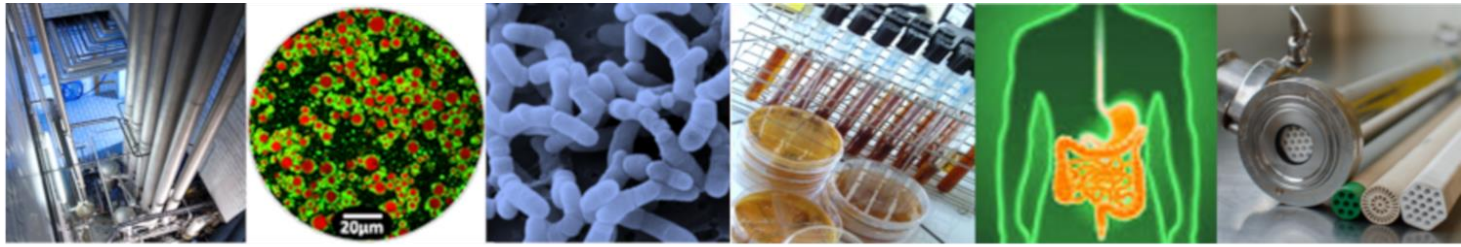
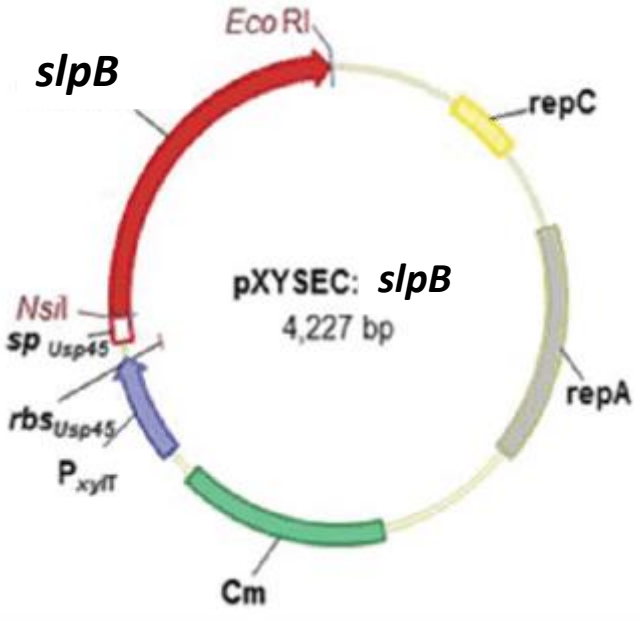
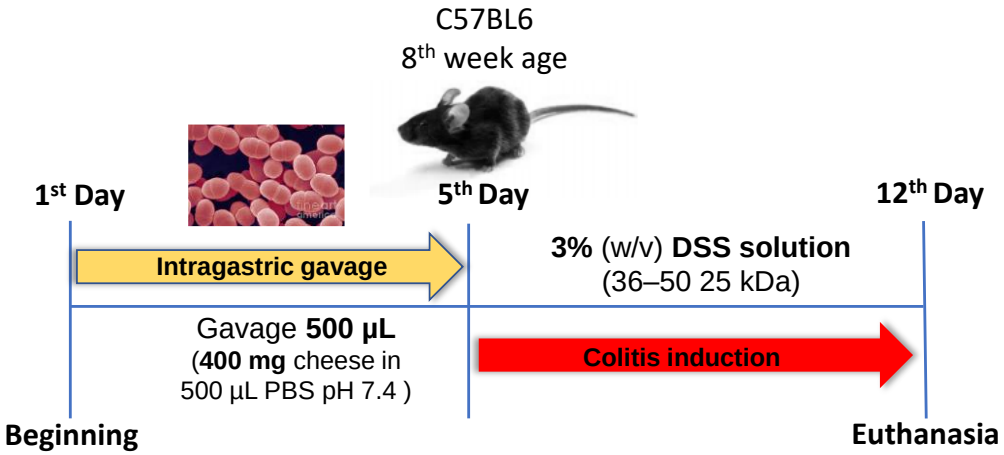
Mutation of SlpB suppresses protection

Heterologous expression of slpB gene



Impact on anti-inflammatory effect





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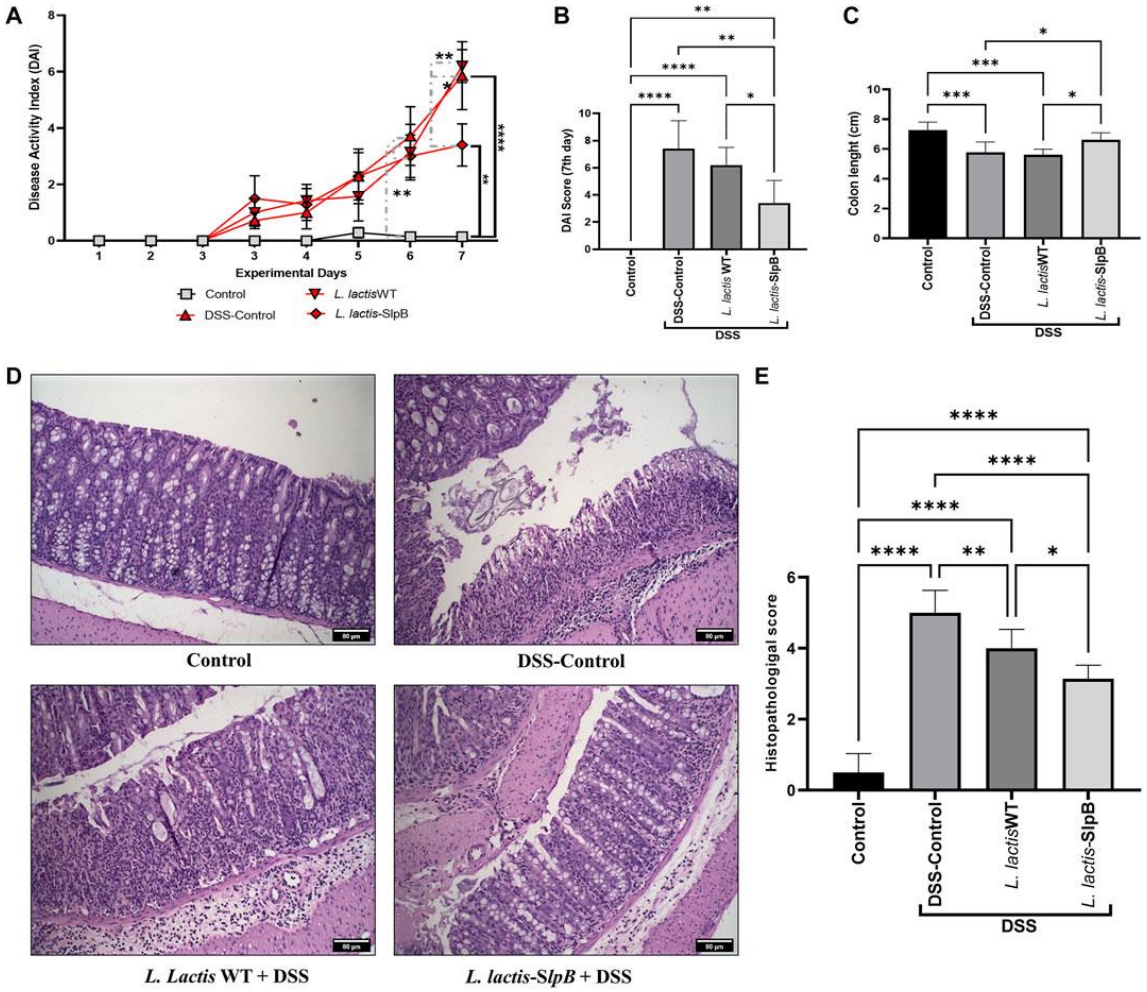
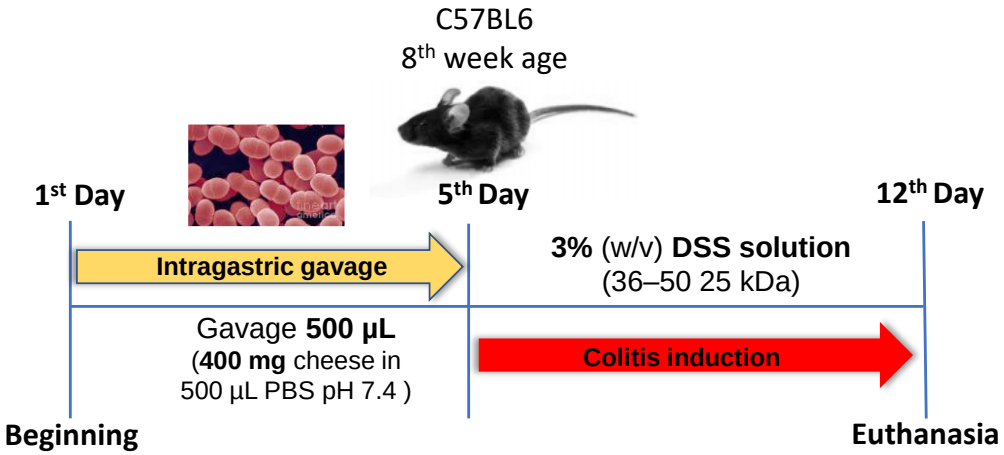
Impact on anti-inflammatory effect





SlpB expression provides *L. lactis* with enhanced anti-inflammatory properties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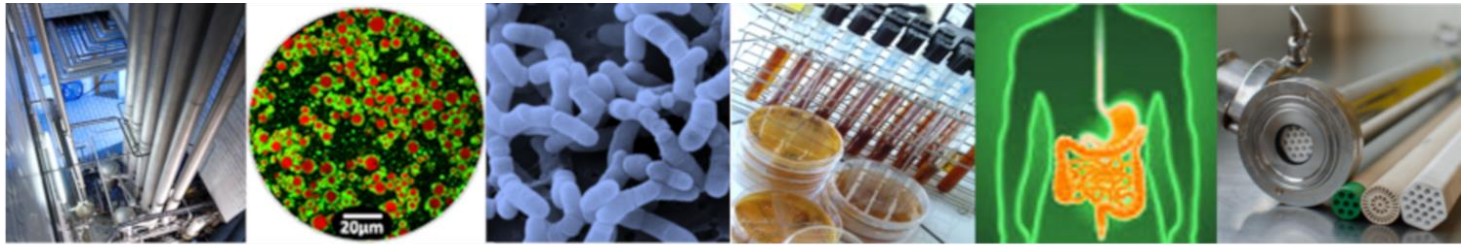
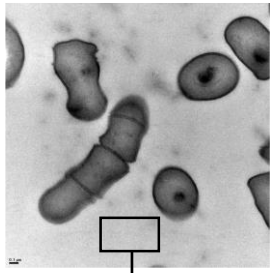
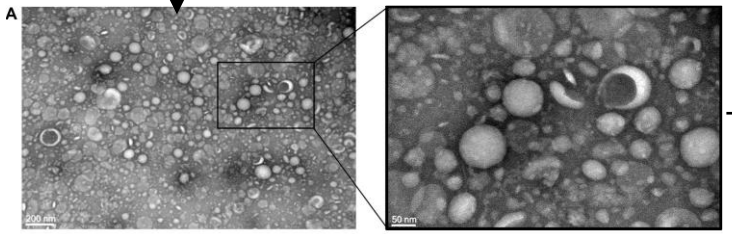
What about Evs ?

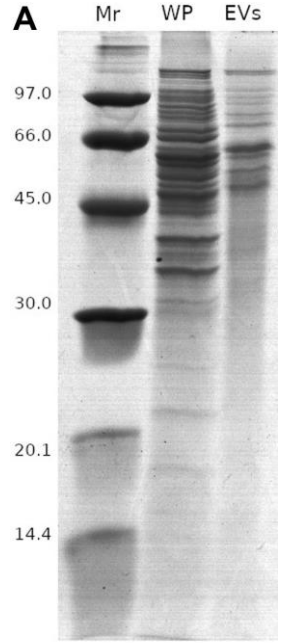


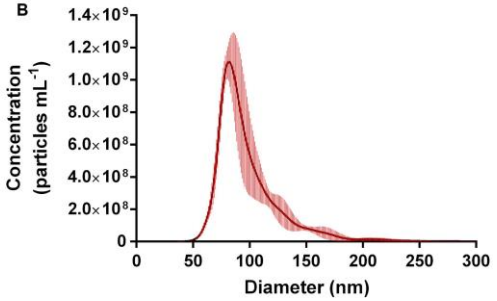
Propionibacteria produce EVs



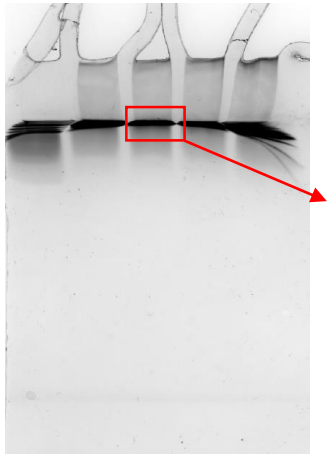


→ SDS solubilisation → SDS PAGE

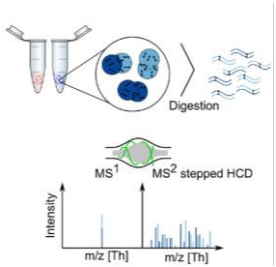




SDS solubilisation → Barely run SDS PAGE



In-gel trypsin digestion



Nano-LC-MS/MS
Q Exactive

INRAE

Propionibacteria immunomodulation
And EVs

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Eric Guédon, INRAE, STLO

STLO

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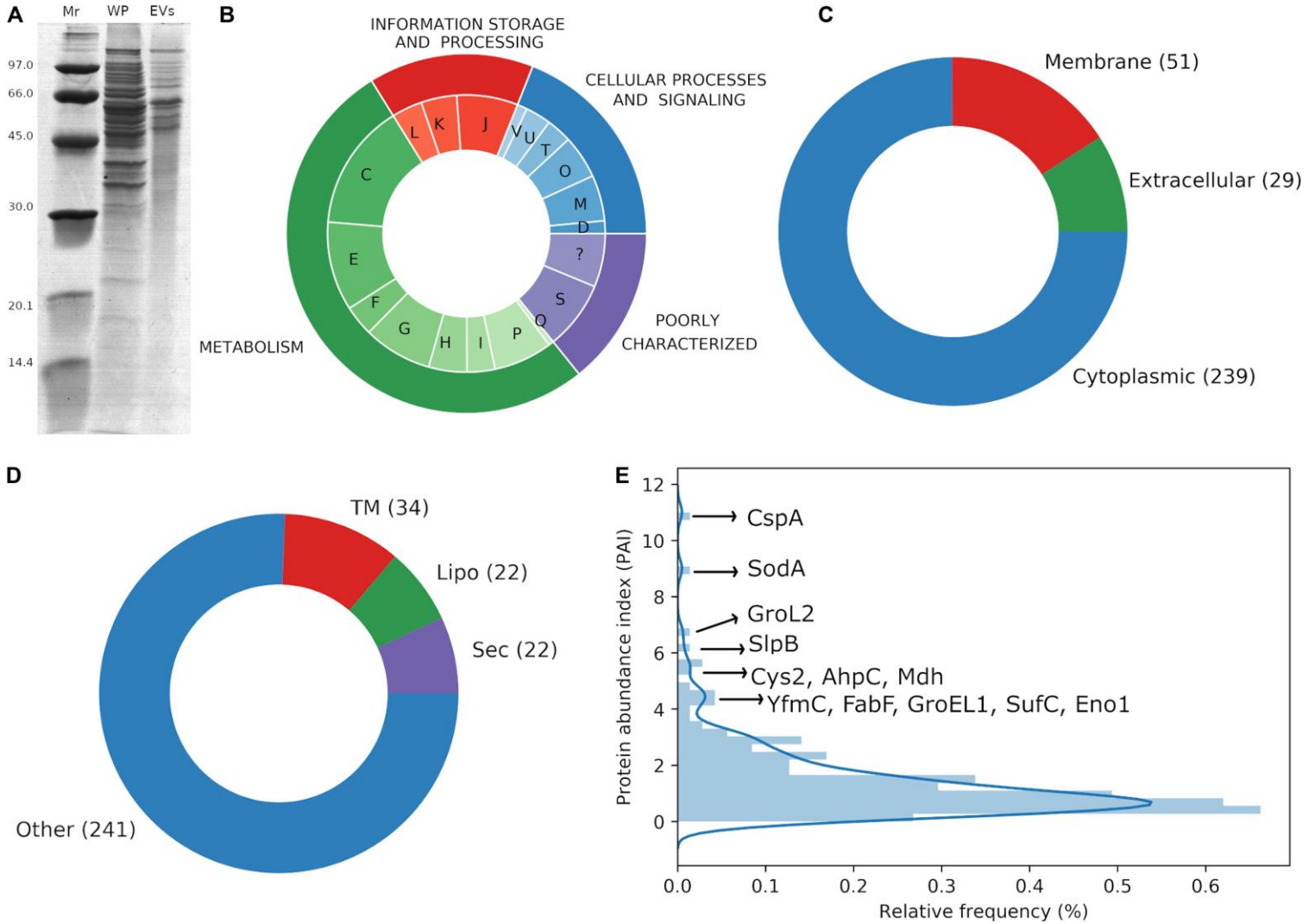
Propionibacteria Evs contain (surface) proteins



INRAE

And the biological activity of these Evs?

Wait for Juliana Laguna

“Health benefits of EVs derived from the probiotic bacterium Propionibacterium freudenreichii”

And for Marine Mantel

“Inhibitor function of bacterial EVs in the epithelial breakdown induced by inflammation: could it be used to treat IBD?”