



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

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Gwénaël Jan. A proteomic analysis of immunomodulatory effects mediated by the probiotic propionibacterium freudenreichii and by its EVs. Journée GO-EVs sur les vésicules extracellulaires, inrae, Oct 2023, Nantes, France. hal-04260177

HAL Id: hal-04260177

<https://hal.inrae.fr/hal-04260177>

Submitted on 26 Oct 2023

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

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[@inrae.fr](mailto:gwenael.jan@inrae.fr)

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





Science & Technology of Milk and Eggs

<http://www6.rennes.inra.fr/stlo>

A multidisciplinary and multiscale approach,
reinforced by two high-calibre facilities:



Dairy Platform



Biological Resource Centre

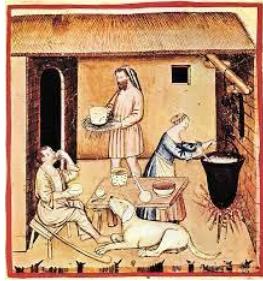
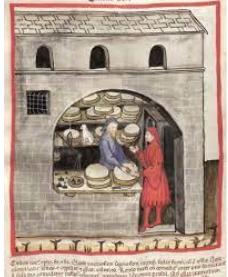


- Structuration / destructuration mechanisms of food matrix:
*from structural characterisation to digestion***
- Dairy processing and cheese making:
*toward sustainable dairy systems***
- Microbial interaction:
*food matrix and host cell***

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



➤ Emmental and other cheeses with holes: a long story



Flavour:



Fruity

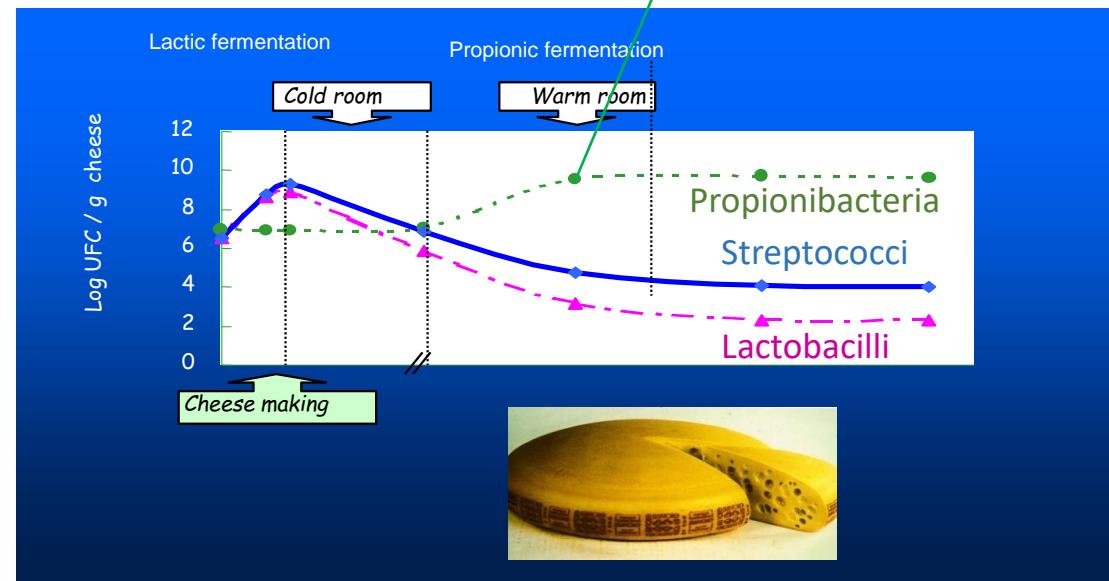


Sour



Picante

Propionate
Acetate
 CO_2
...Esters



➔ The Fitz equation (1878)

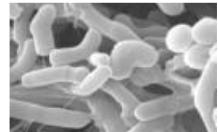


➔ The isolation of dairy propionibacteria by von Freudenreich and Orla-Jensen (1907)

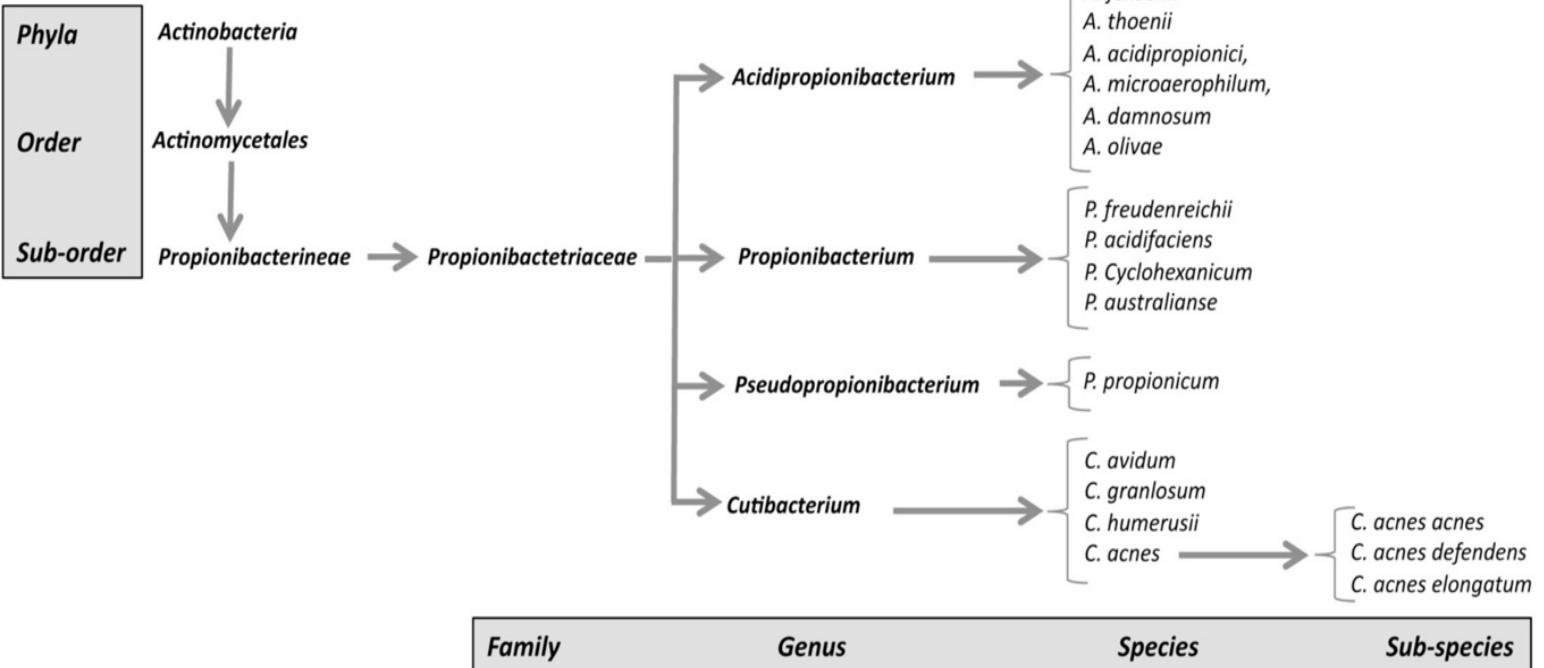


INRAe

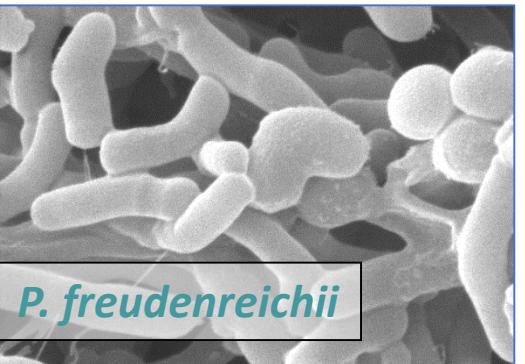
Propionibacteria immunomodulation
And EVs



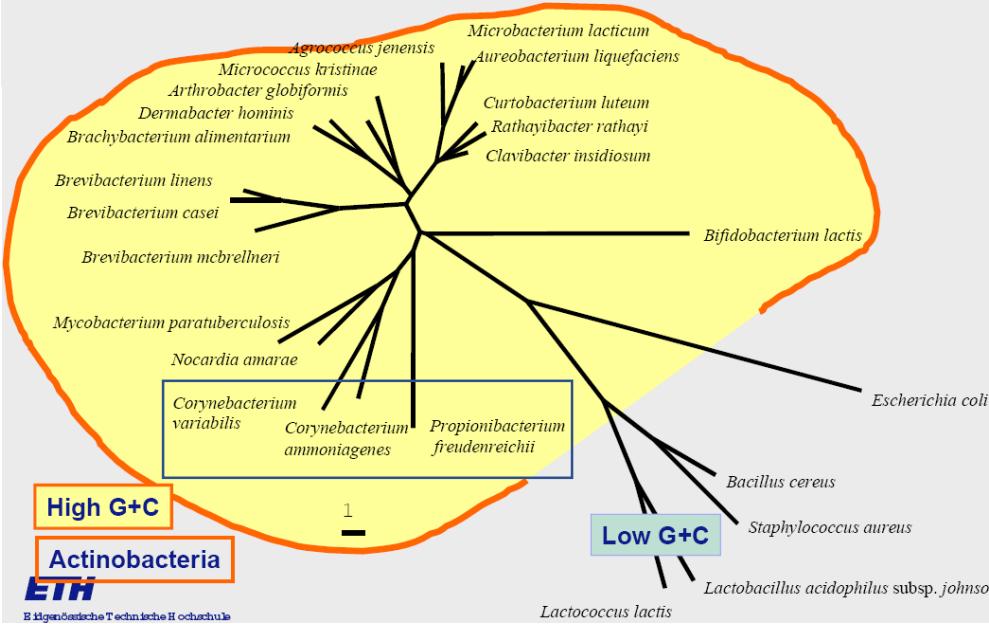
> Propionibacteria, in a few words



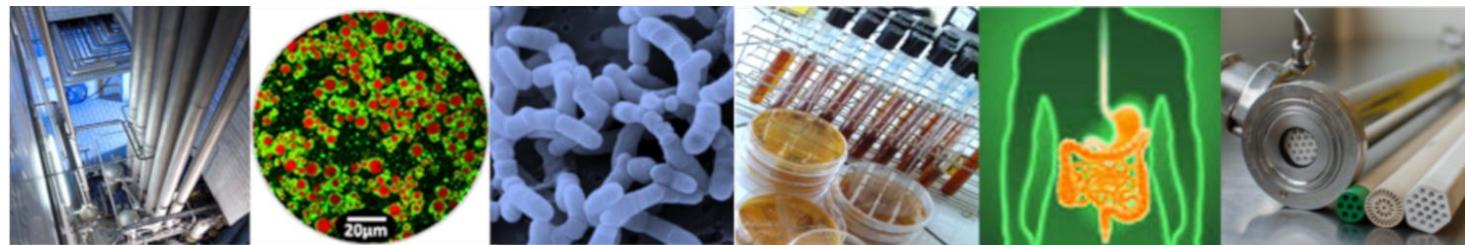
Gram +
pleiomorphic
anaerobic to microaérophilic
non motiles et non sporulated



Phylogenetic tree of the class Actinobacteria based on 16S rDNA



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



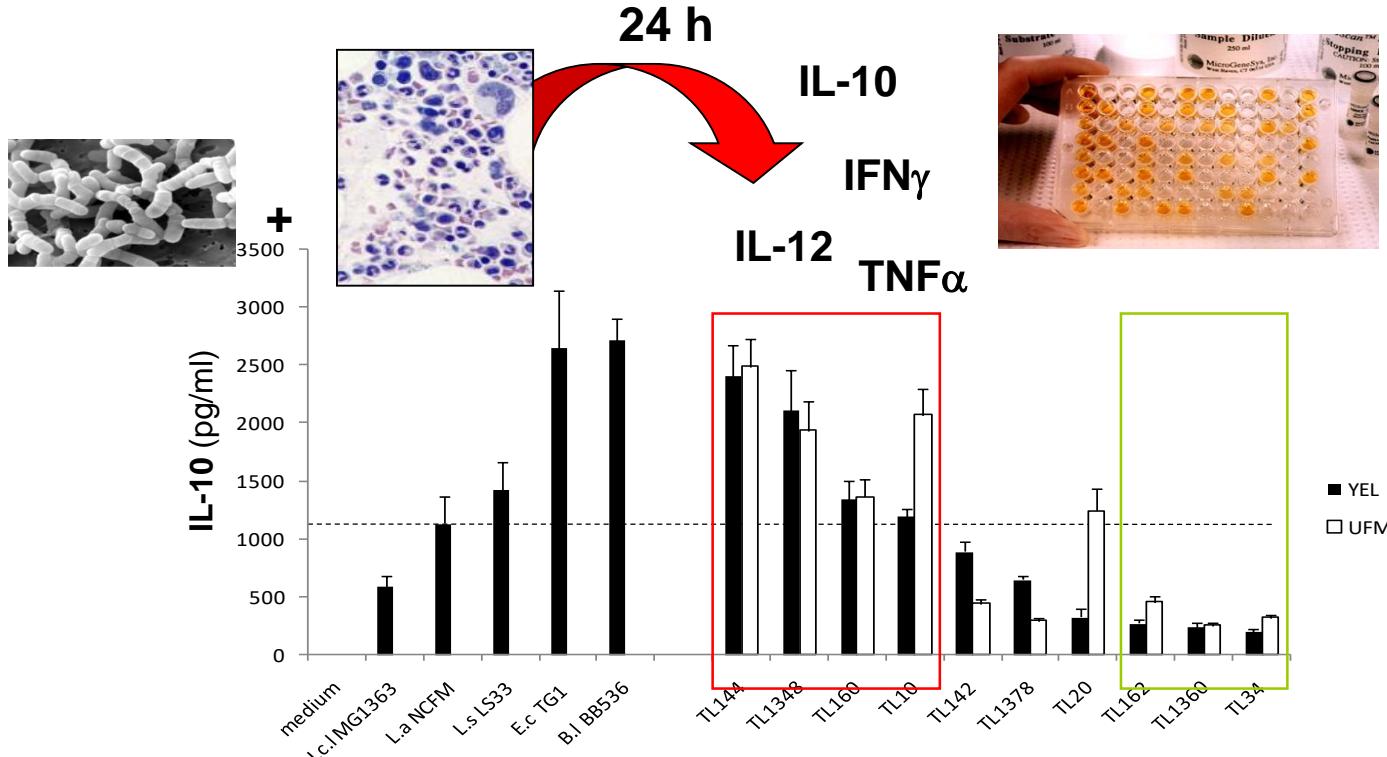
➤ Cytokine stimulation on human PBMCs (mononucleated leucocytes from donors)



Bruno Pot



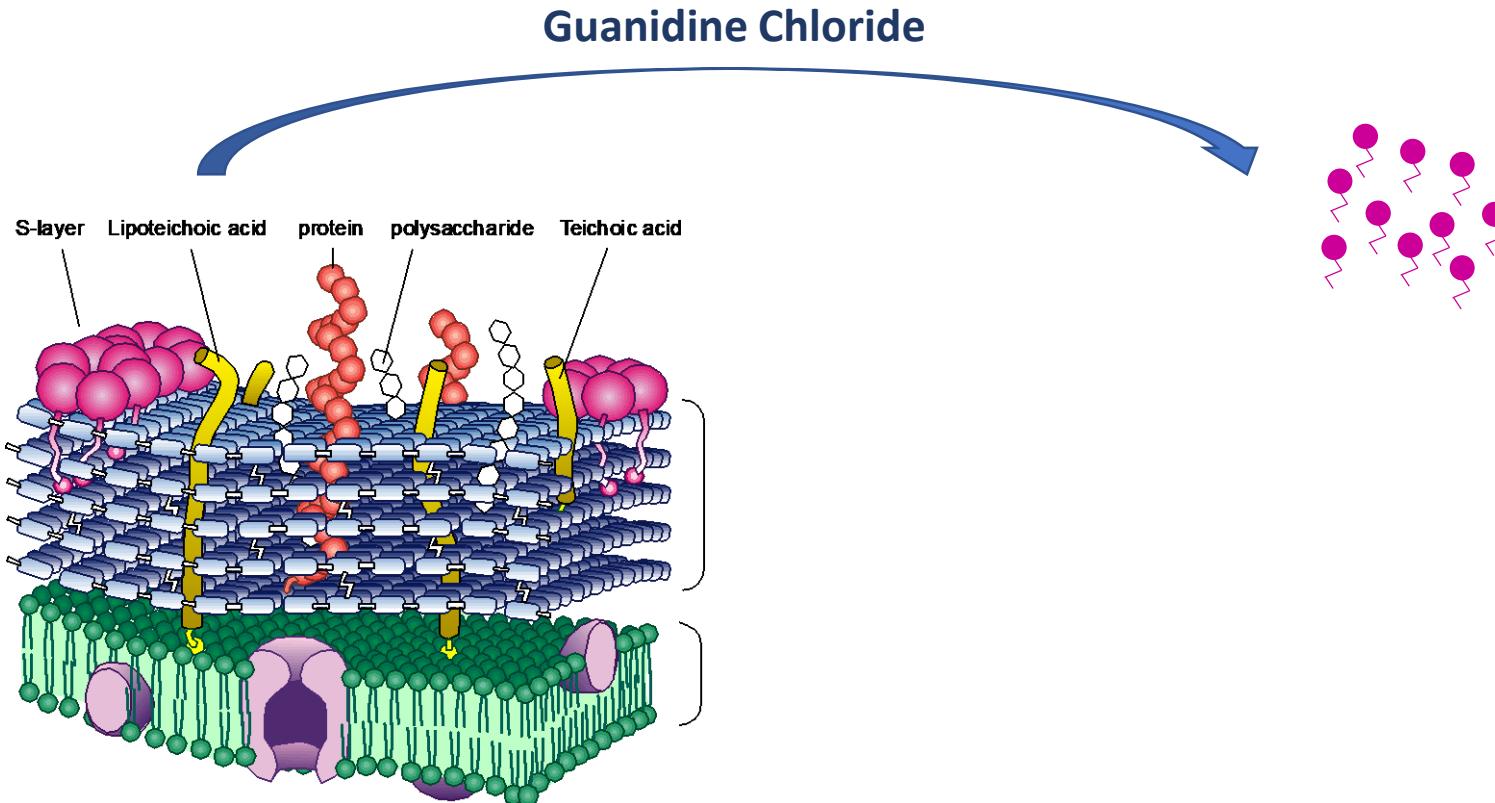
Benoit Foligné



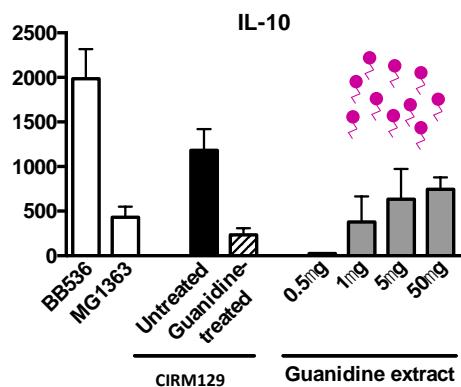
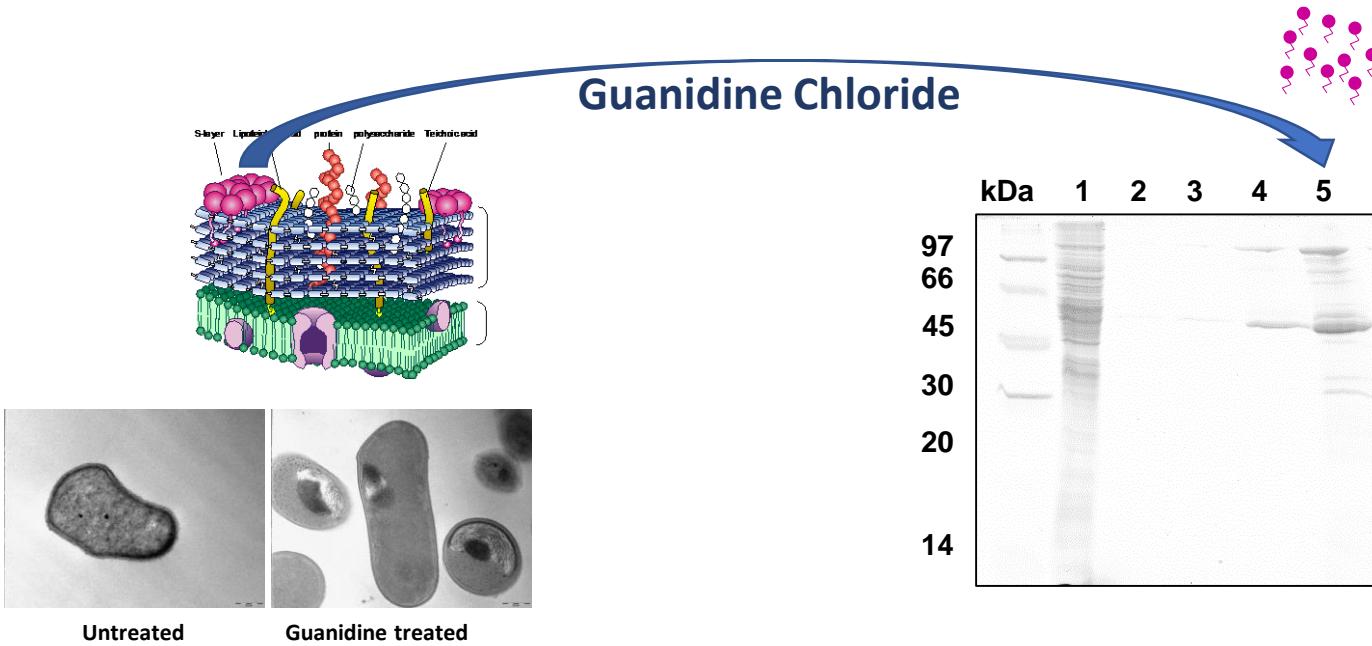
The best anti-inflammatory strains

The less anti-inflammatory strains

> Cytokine stimulation: surface layer proteins (SLP) involved



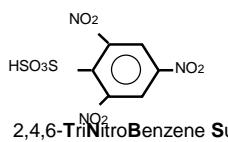
➤ A key role of surface proteins



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



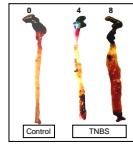
> Propionibacteria: prevention of TNBS-induced colitis in mice



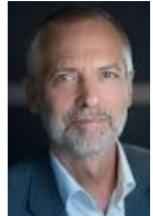
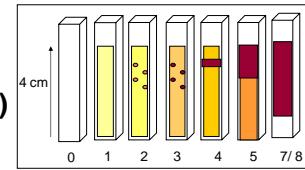
2,4,6-TriNitroBenzene Sulfonic acid (TNBS)



Acute colitis



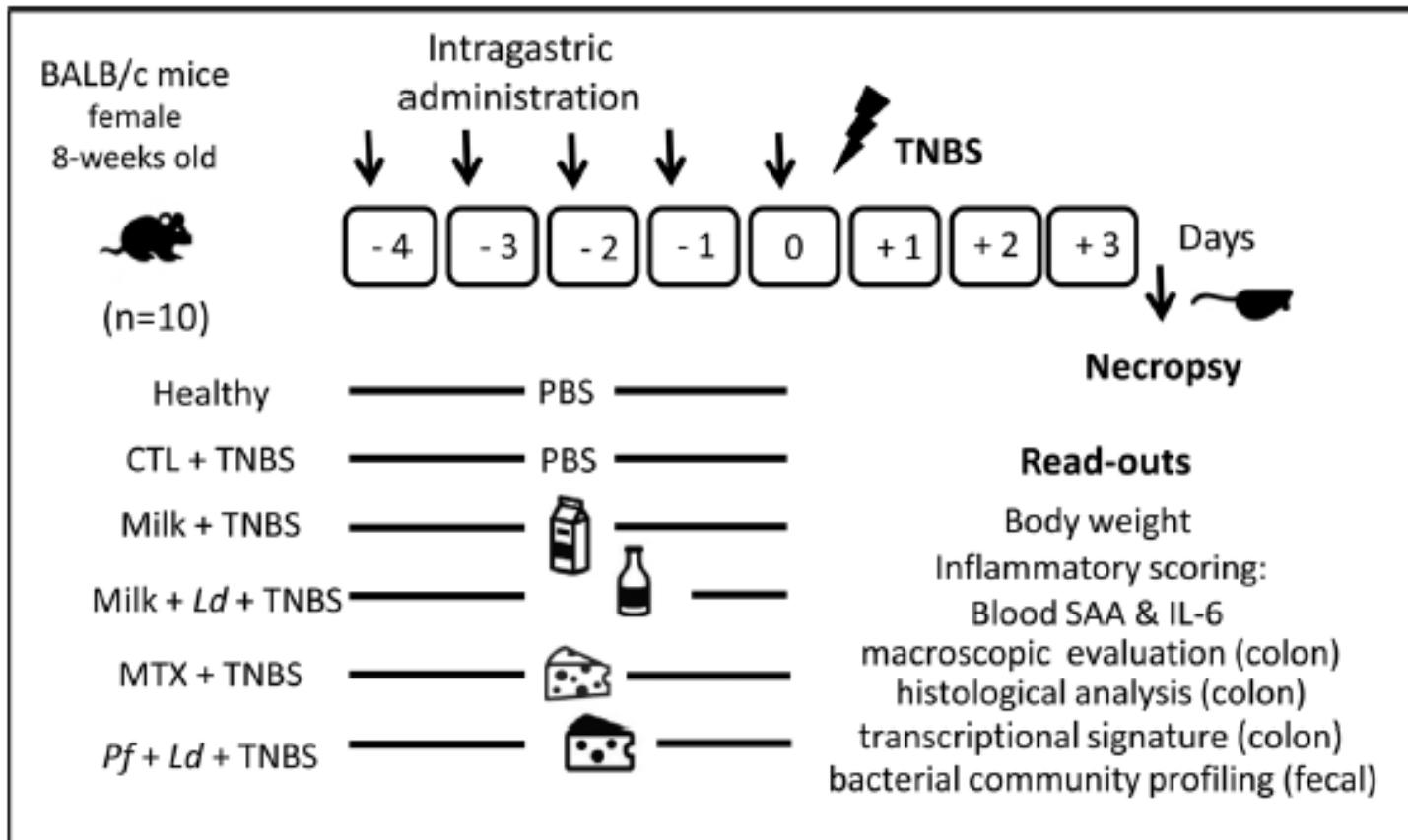
Macroscopic score (Wallace)



Bruno Pot



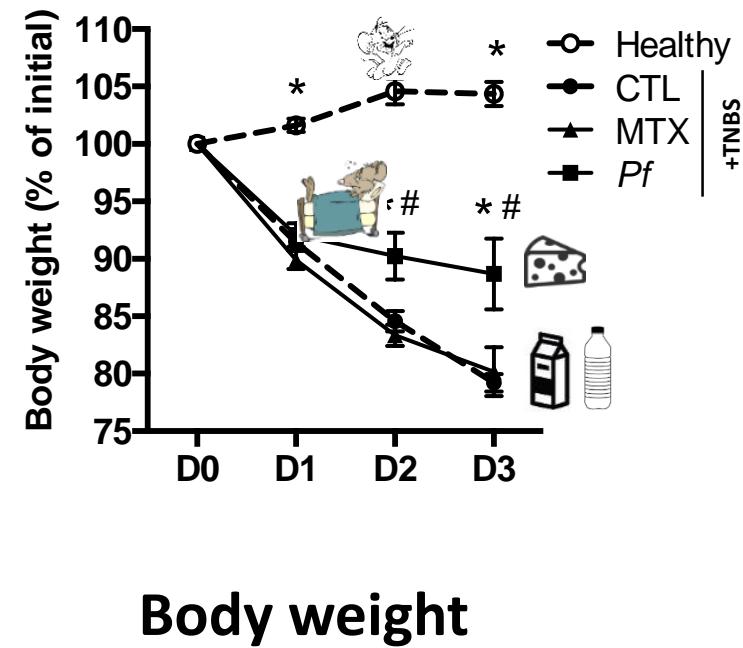
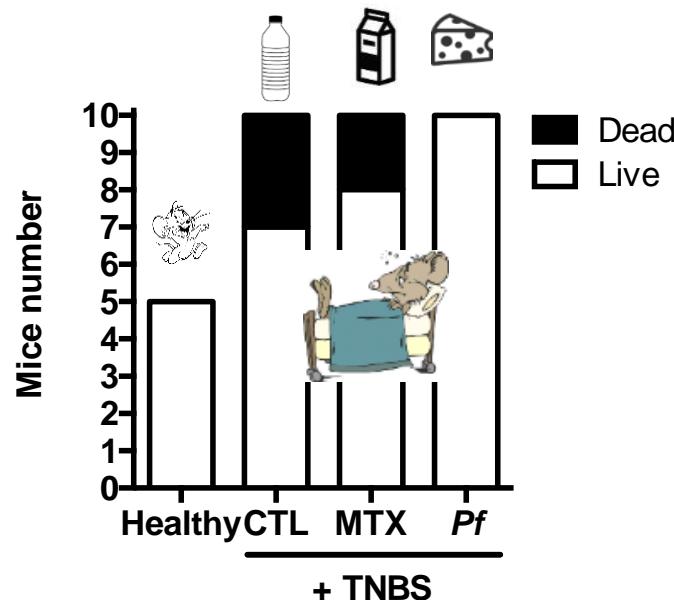
Benoit Foligné



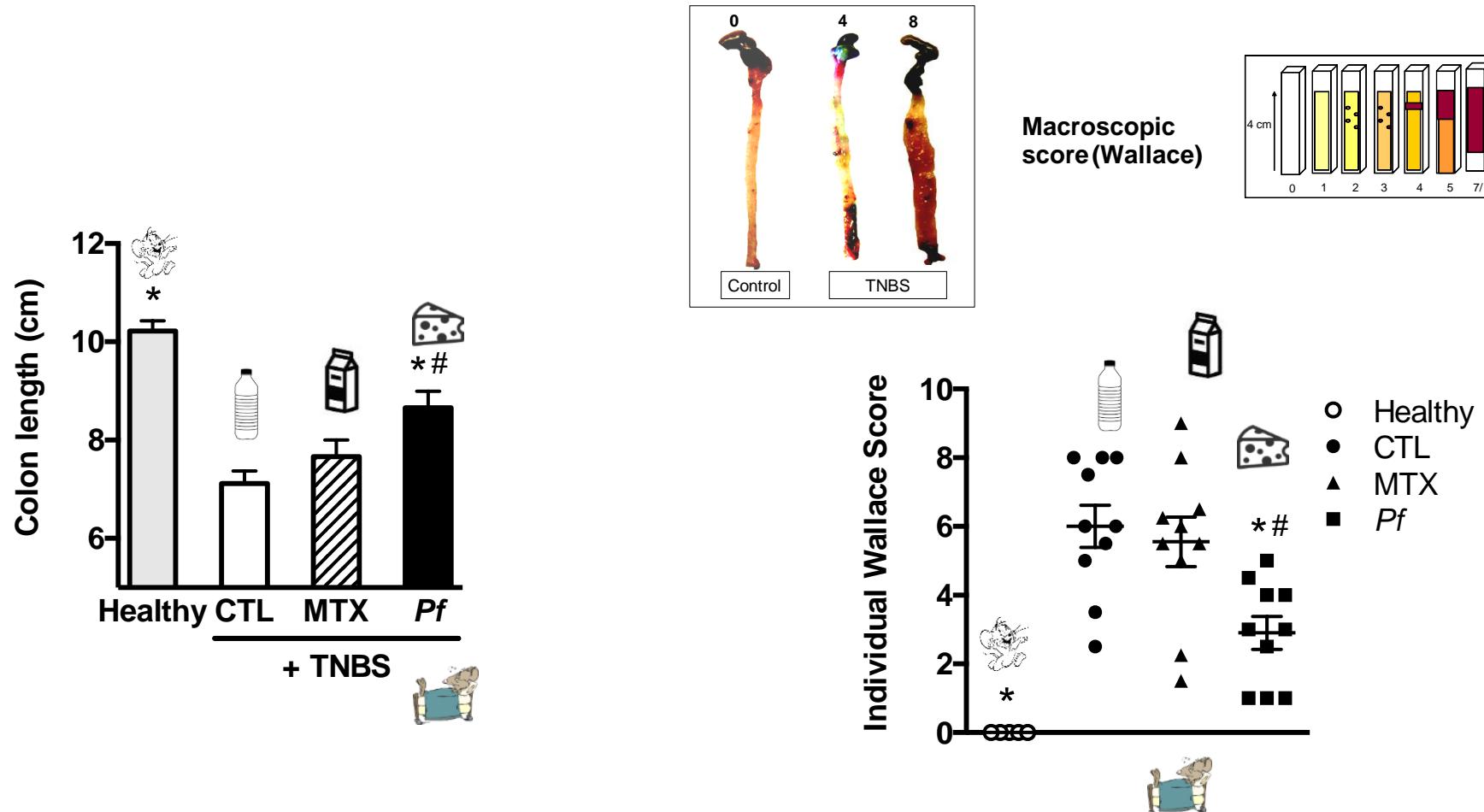
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Propionibacteria immunomodulation
And EVs

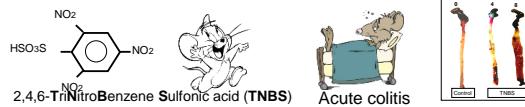
> Propionibacteria: prevention of TNBS-induced colitis in mice



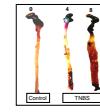
> Propionibacteria: prevention of TNBS-induced colitis in mice



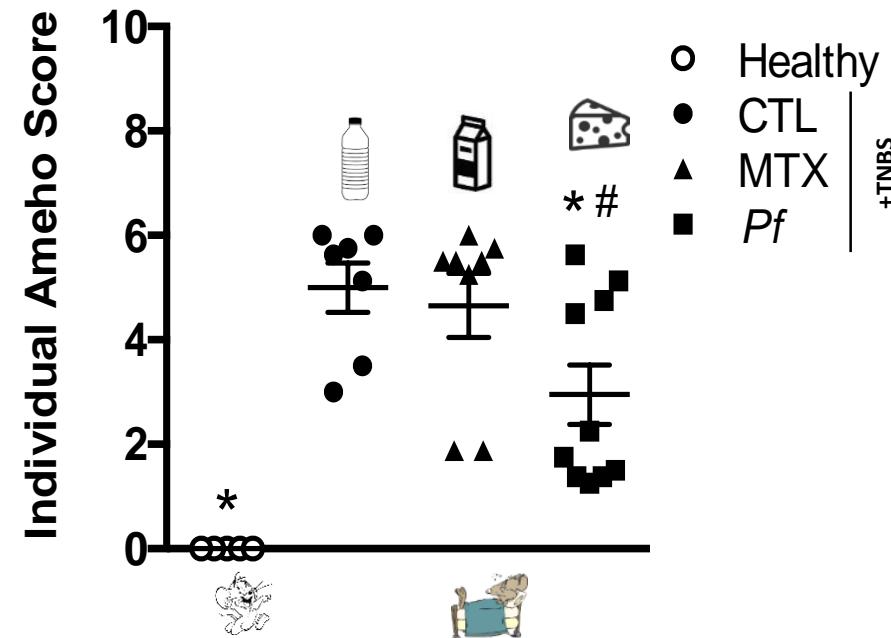
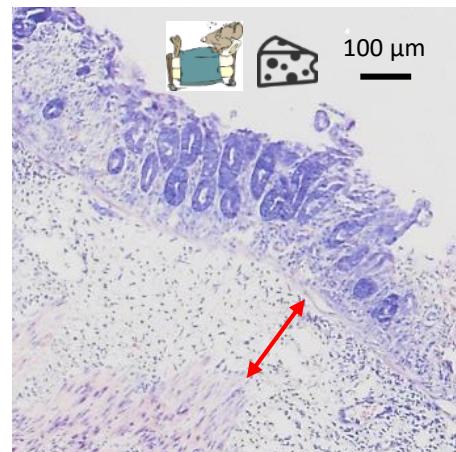
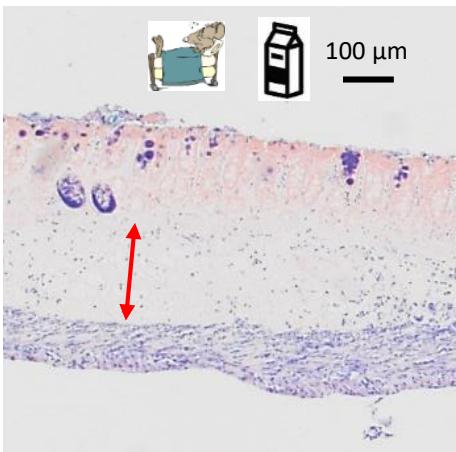
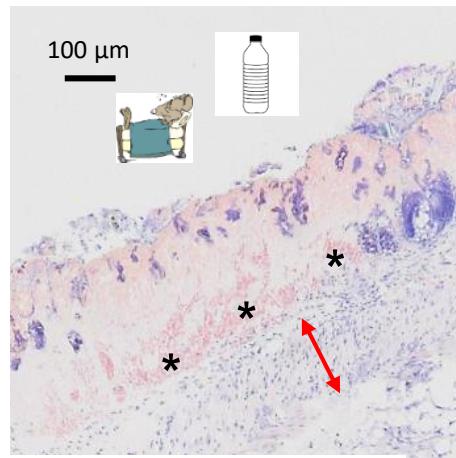
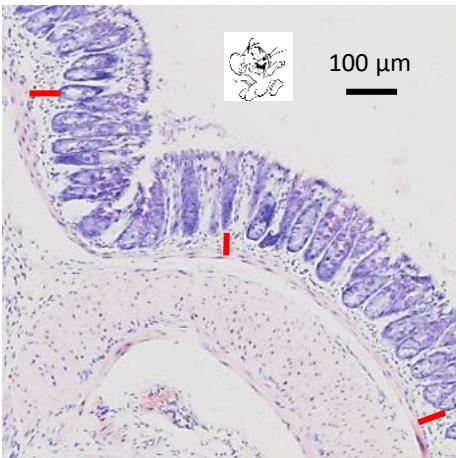
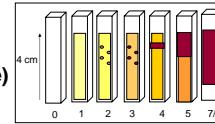
> Propionibacteria: prevention of TNBS-induced colitis in mice



Acute colitis

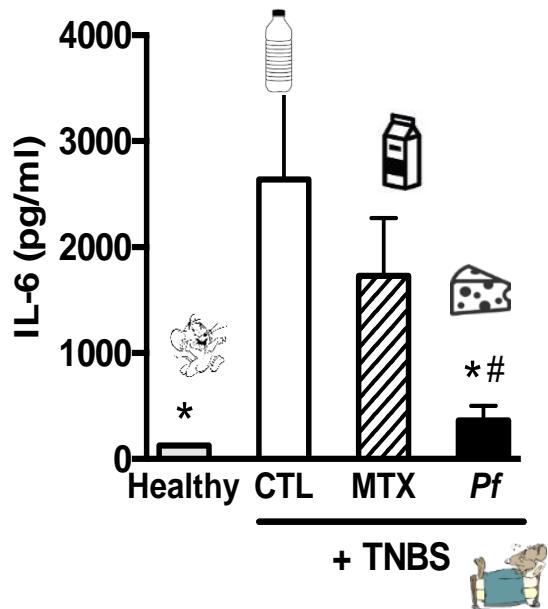


Macroscopic score (Wallace)

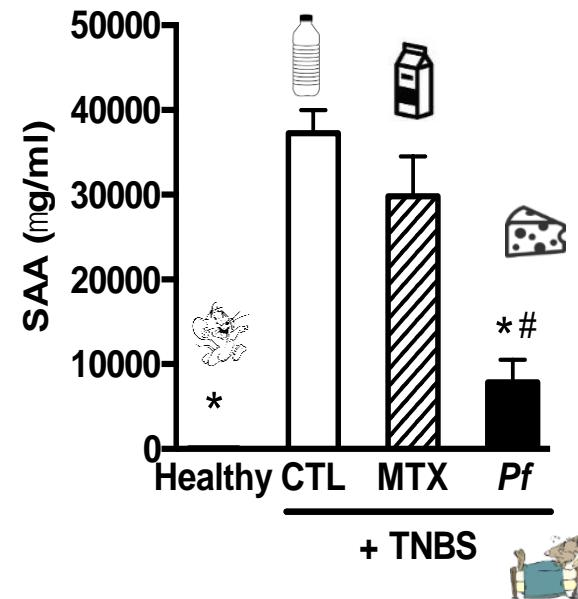


➤ Propionibacteria: prevention of TNBS-induced colitis in mice

Blood inflammation markers



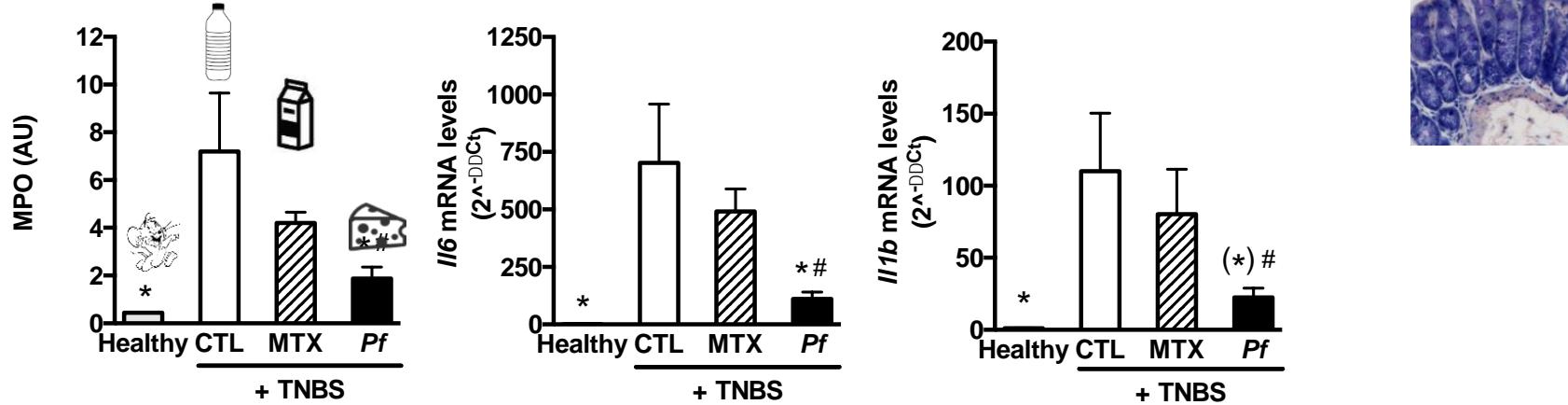
Interleukin 6



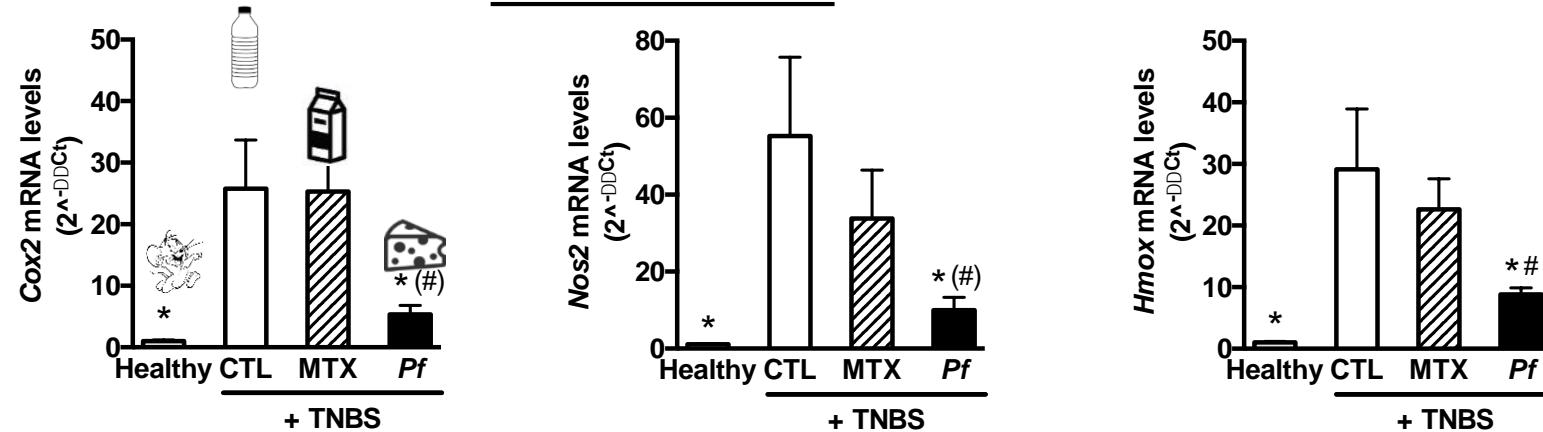
Serum Amyloid A

Propionibacteria: prevention of TNBS-induced colitis in mice

Colonic inflammatory markers

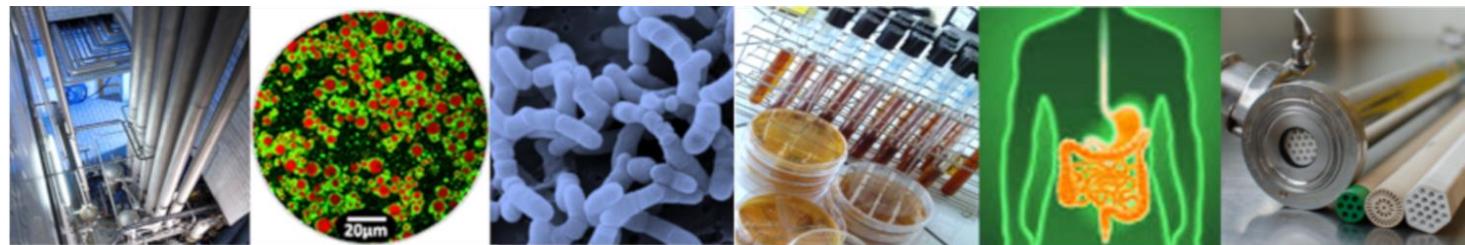


Colonic oxidative stress markers

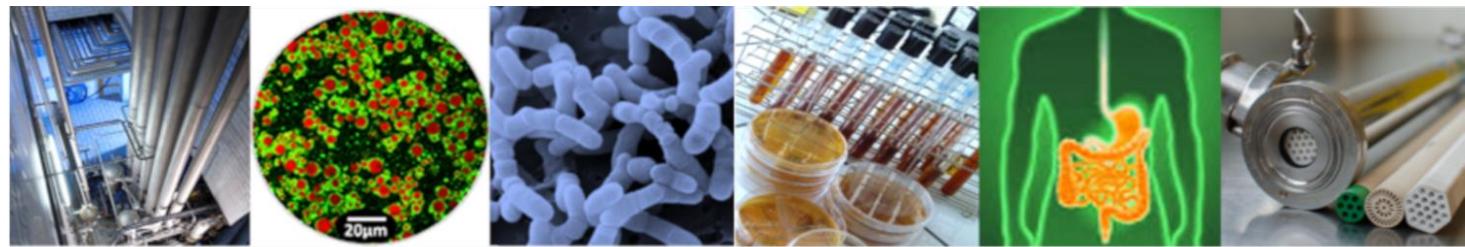


* p<0.05: vs CTL
p<0.05: vs MTX

> How does it work?



> Comparative proteomics related to IL-10 induction

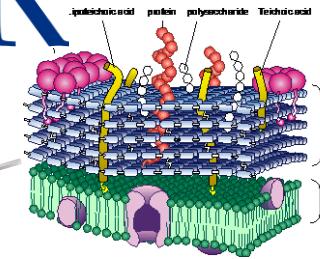


Looking for correlations

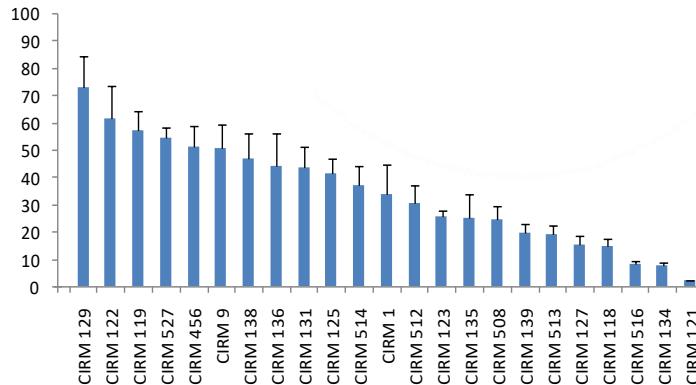


Agence Nationale de la Recherche

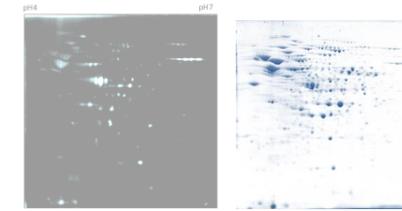
ANR



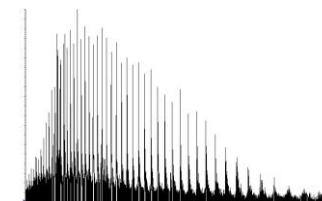
Surfaceome



In vitro : IL-10



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julien.jardin@rennes.inra.fr

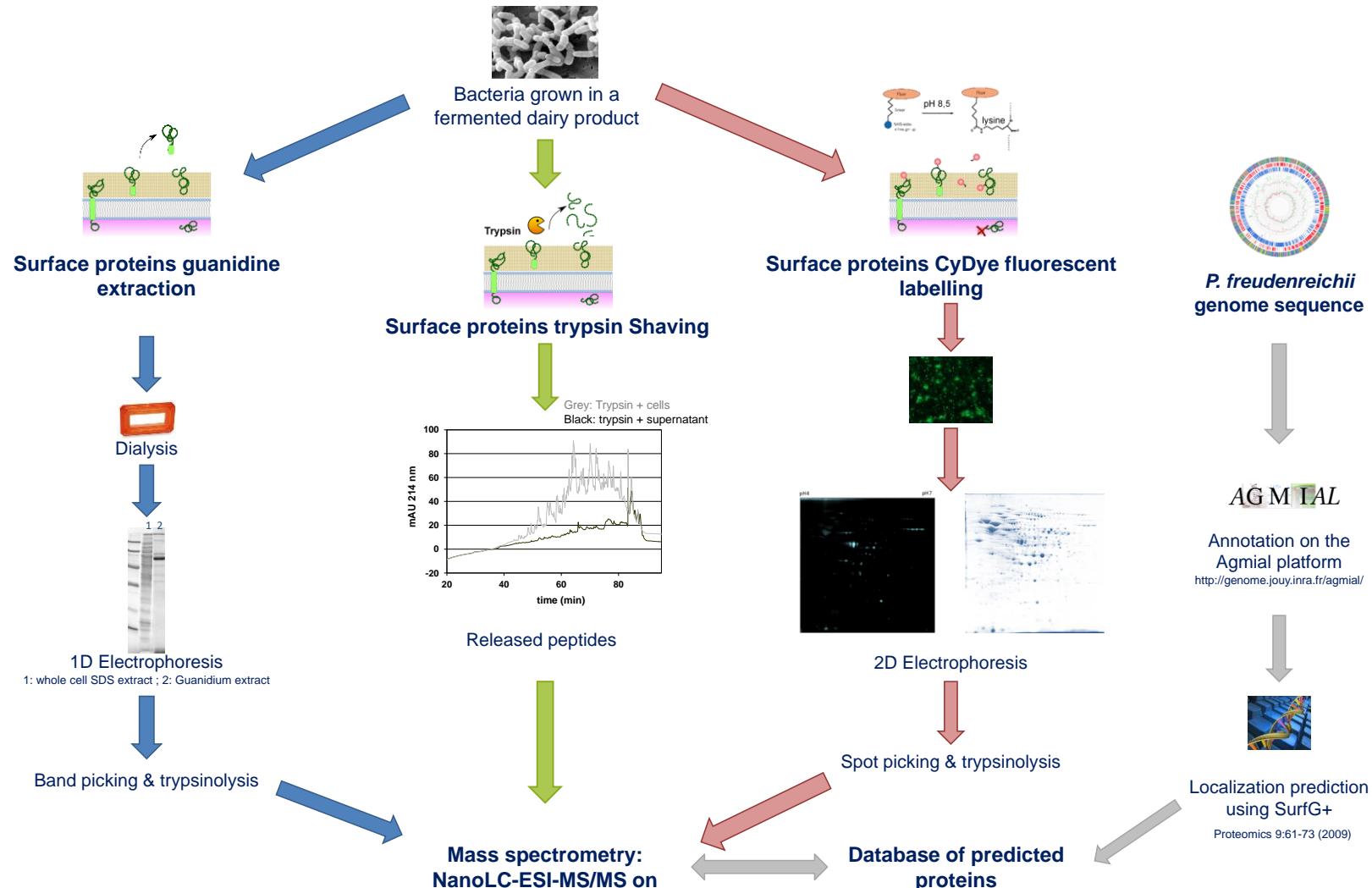


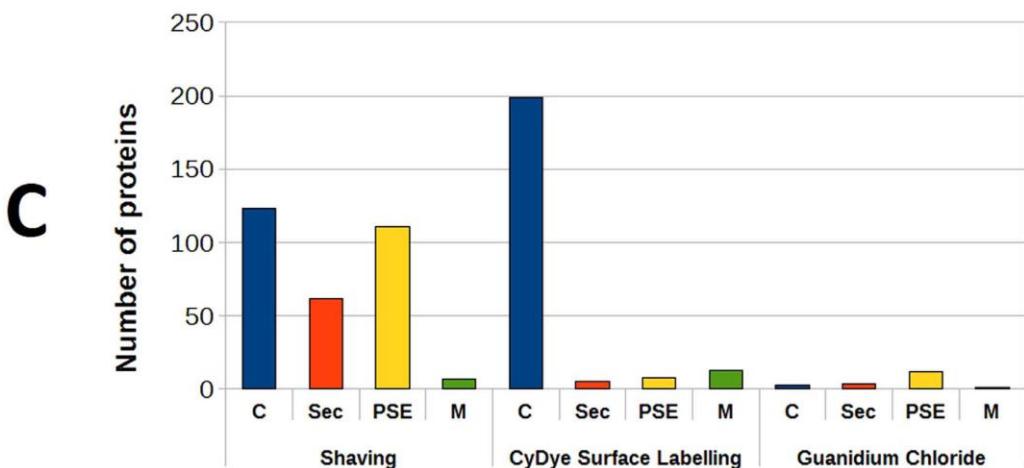
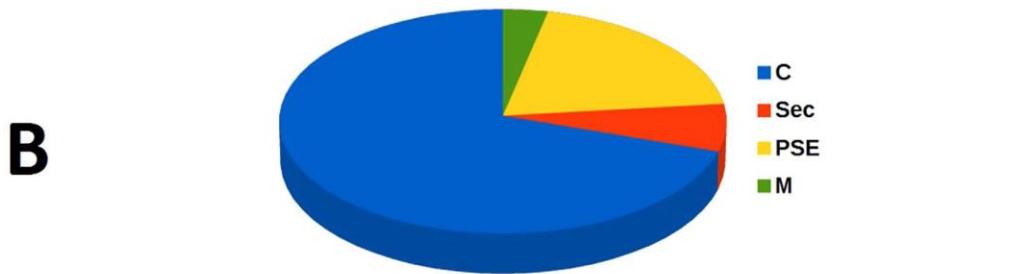
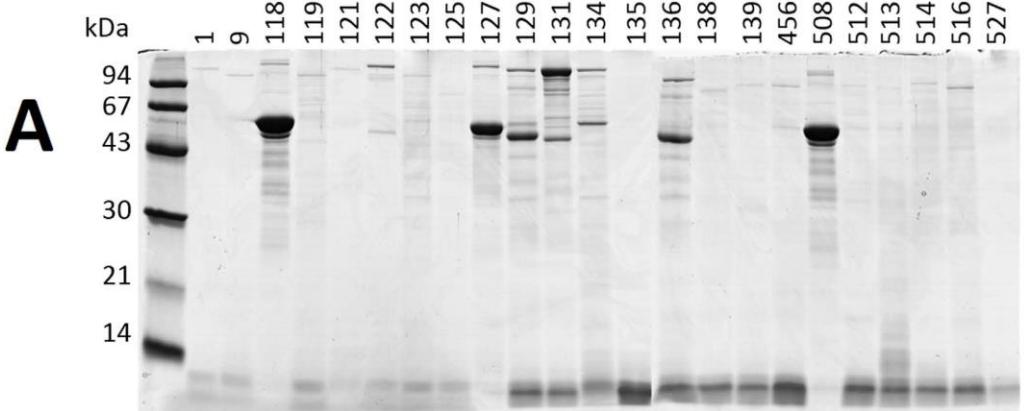
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Propionibacteria immunomodulation
And EVs



The surface proteomic approach

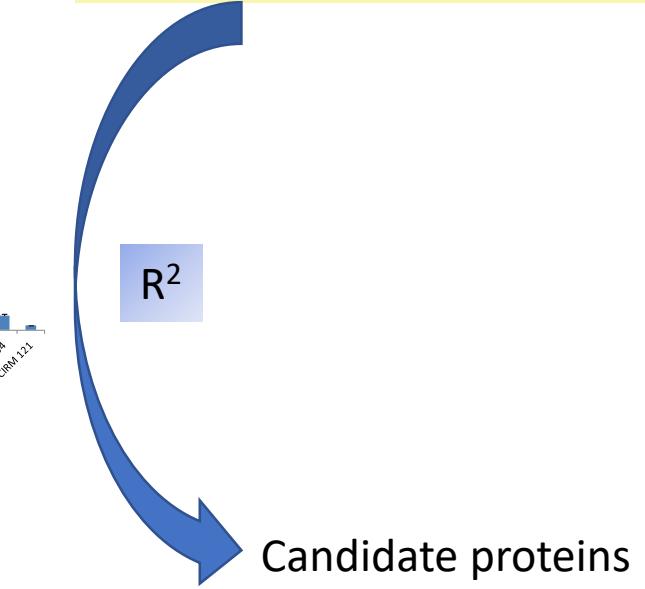
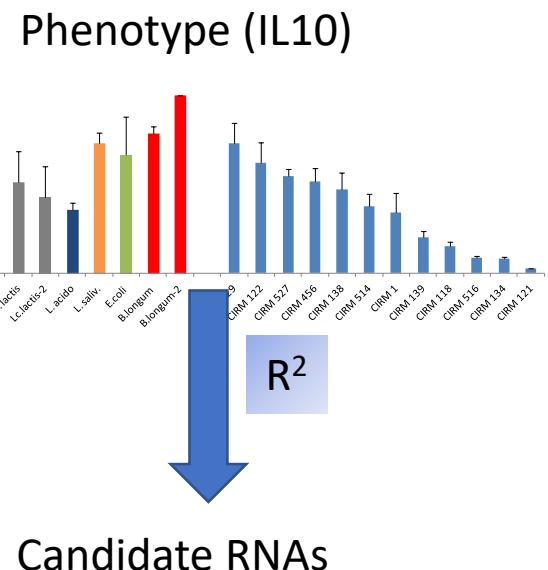
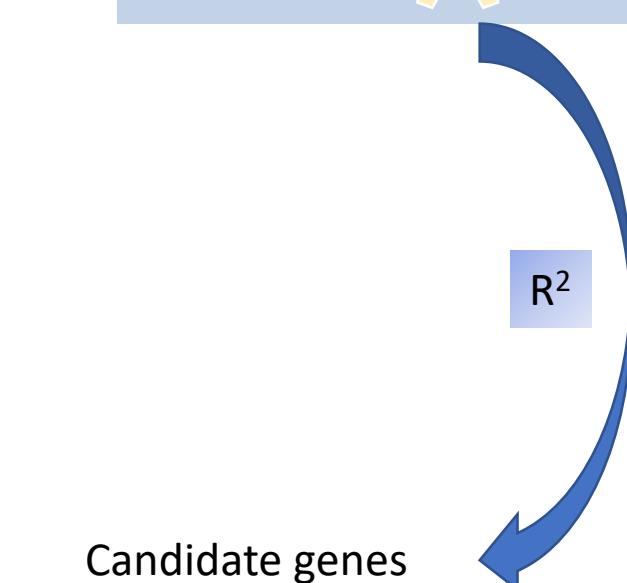
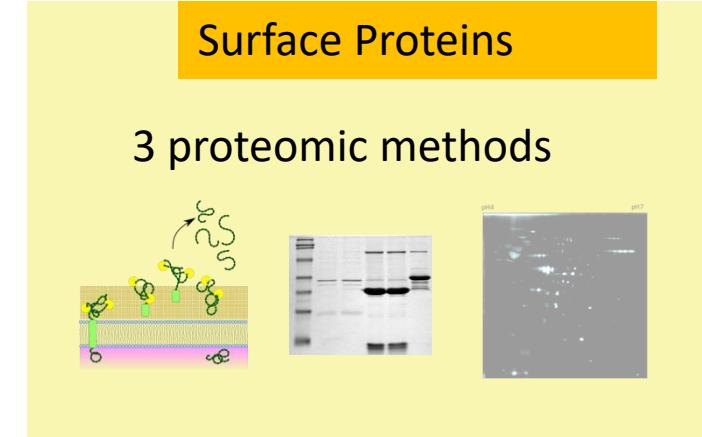
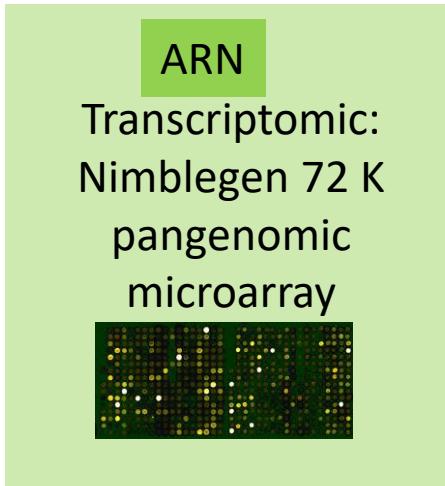
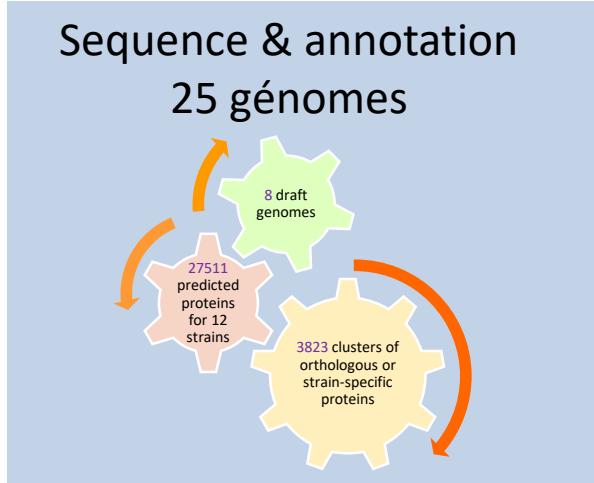




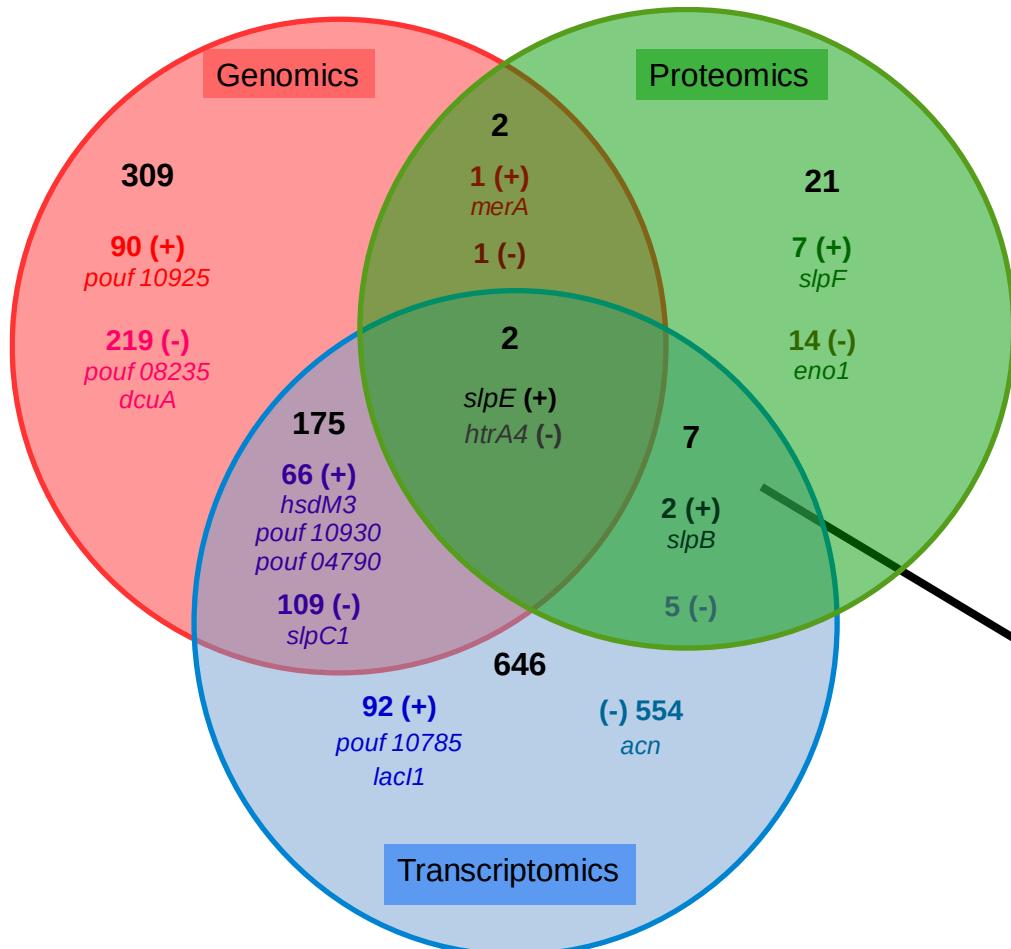
➤ **Diversity of *Propionibacterium freudenreichii* surface proteome, distribution into *in silico* predicted localisations.**

(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



➤ Treating correlations : statistics

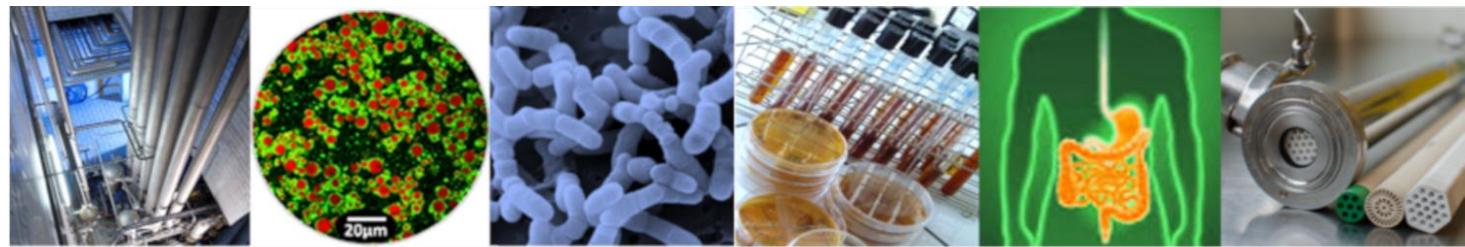


Mahendra.Mariadassou@jouy.inra.fr

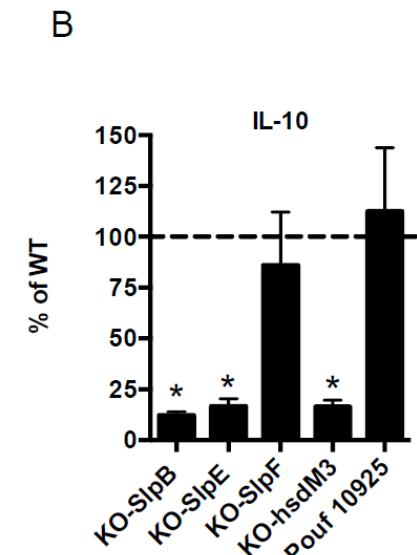
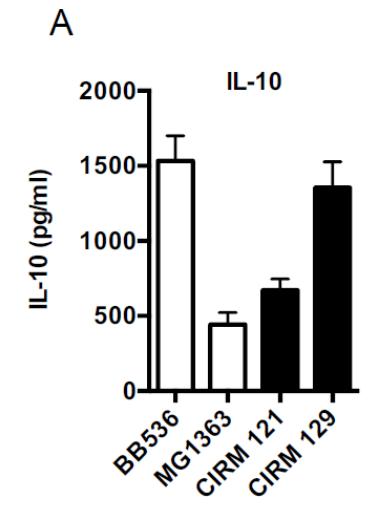
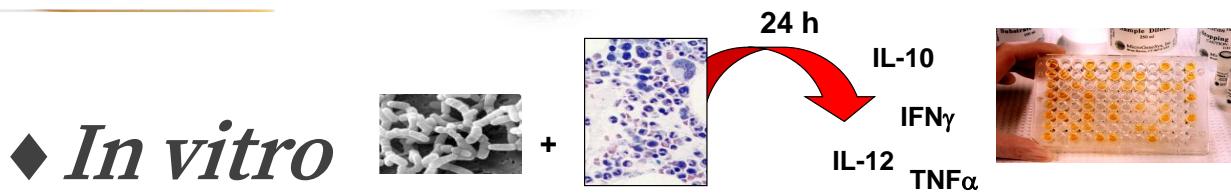
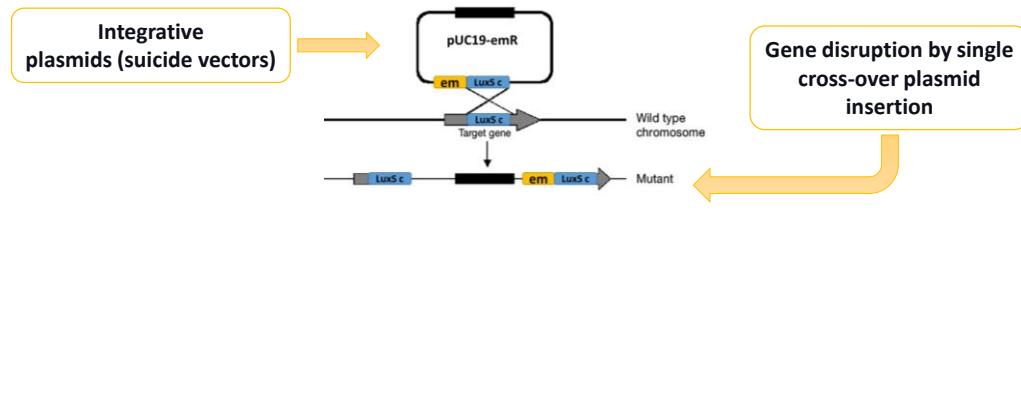
➤ Surface Layer Proteins

➤ A total of 45 genes inactivated

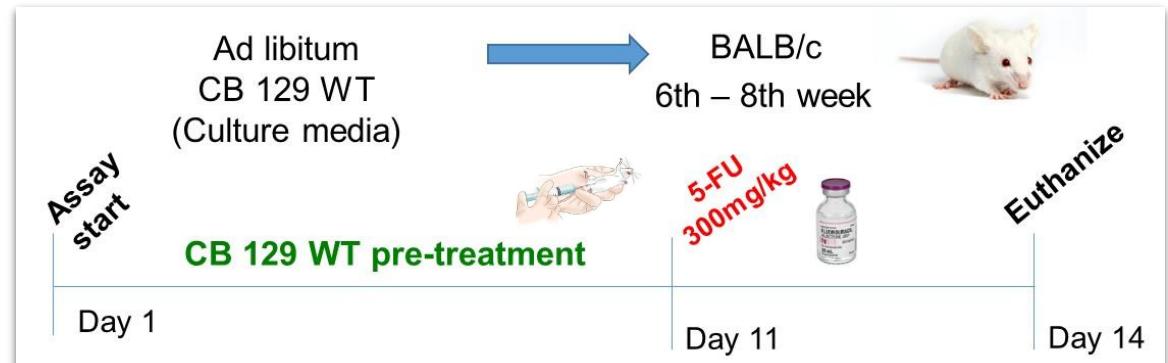
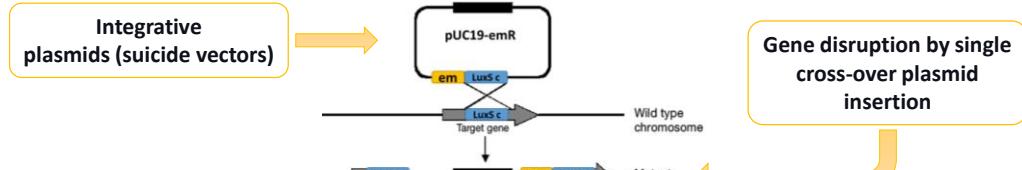
➤ Mutational inactivation of surface layer protein genes



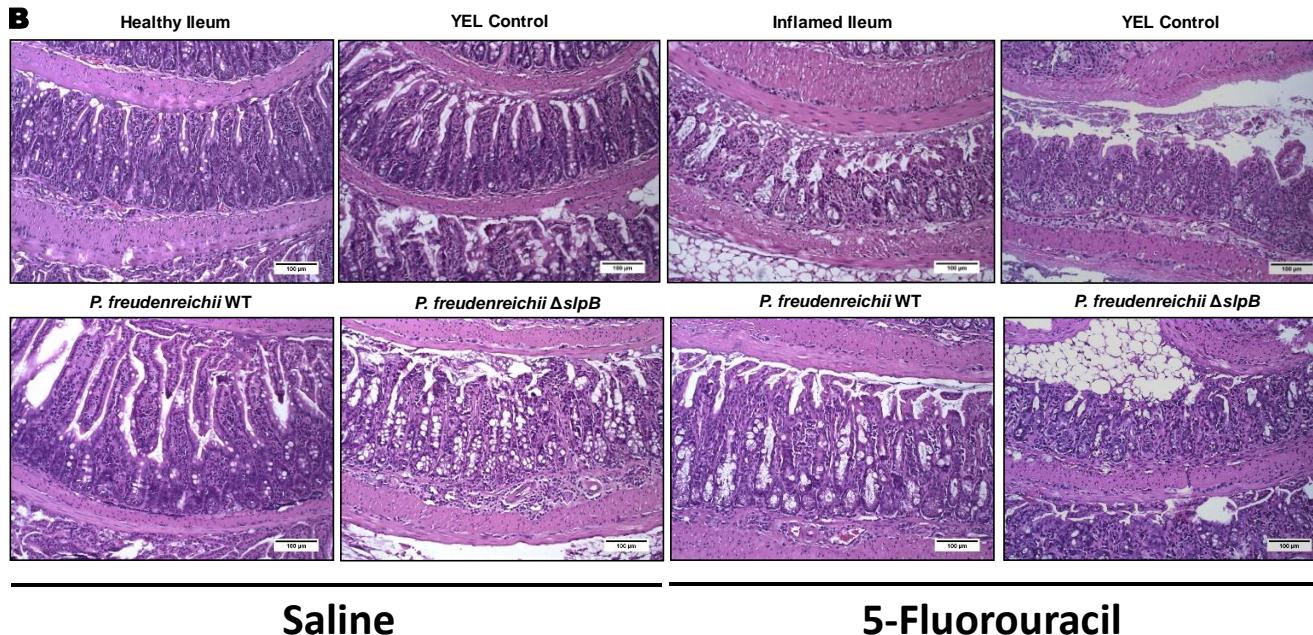
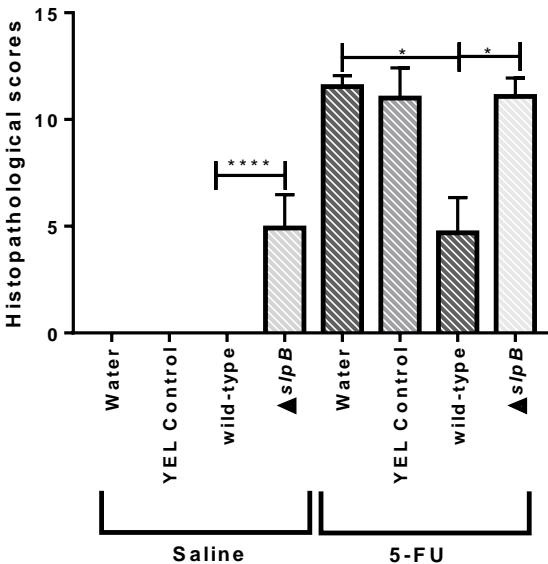
> Impact on IL-10 induction



➤ Impact on anti-inflammatory effect



A



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➤ Mutation of SlpB suppresses protection

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Propionibacteria immunomodulation
And EVs



U F *m* G
UNIVERSIDADE FEDERAL
DE MINAS GERAIS

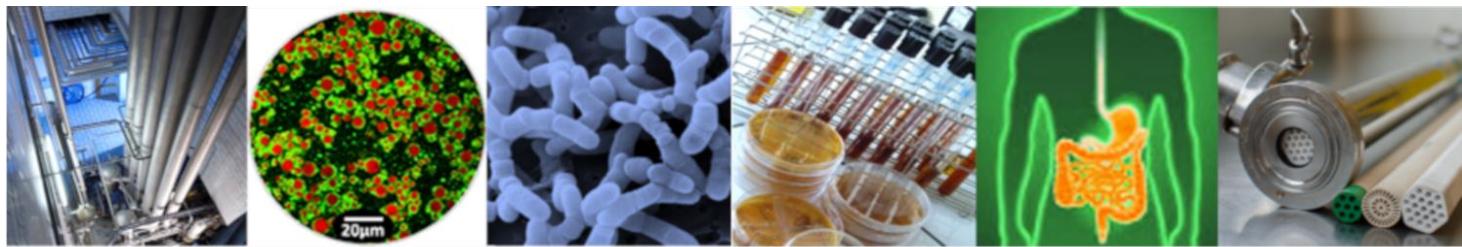
LGCM
Laboratório de Genética
Celular e Molecular

STLO

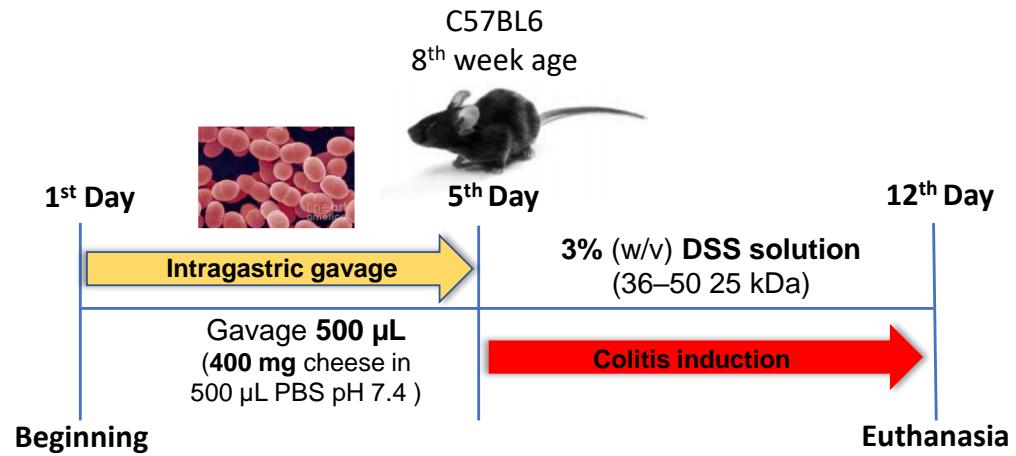
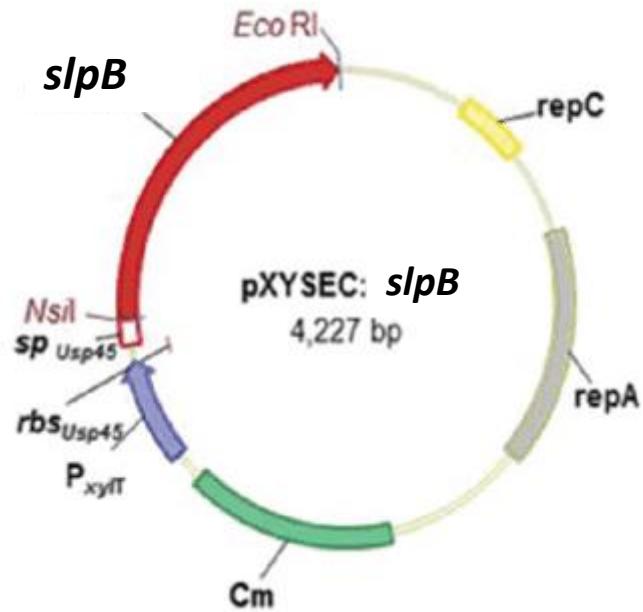
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> Heterologous expression of slpB gene

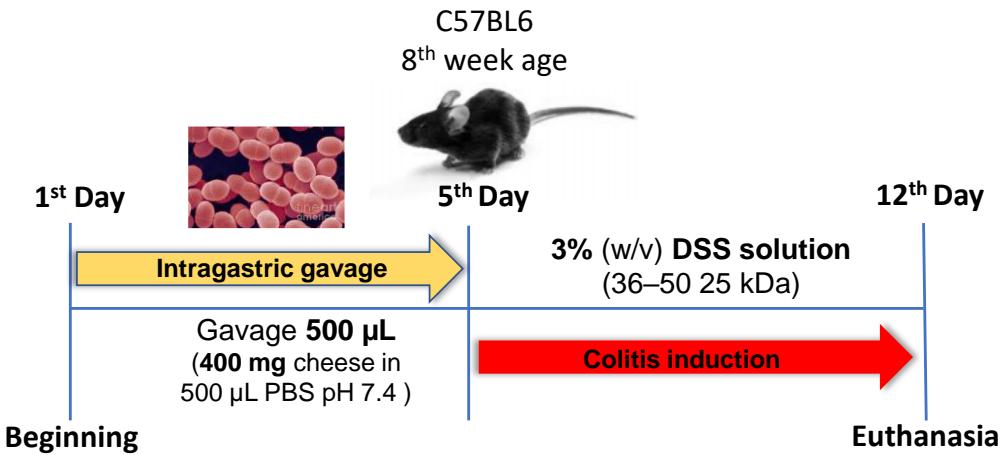
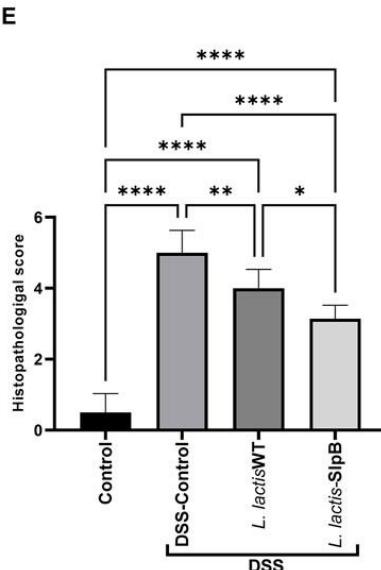
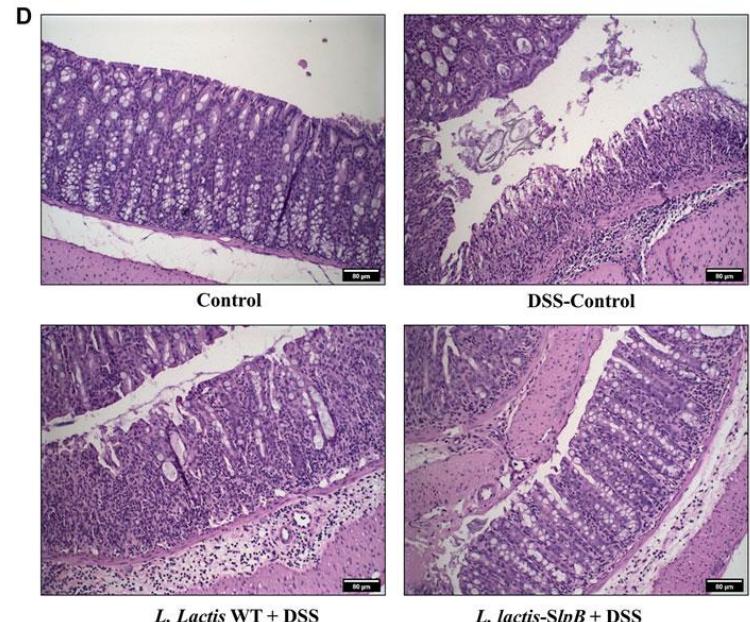
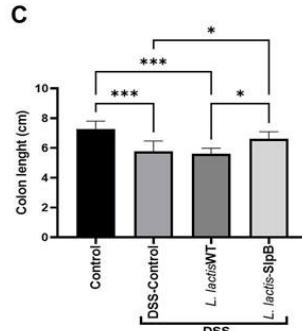
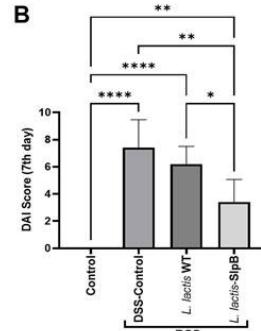
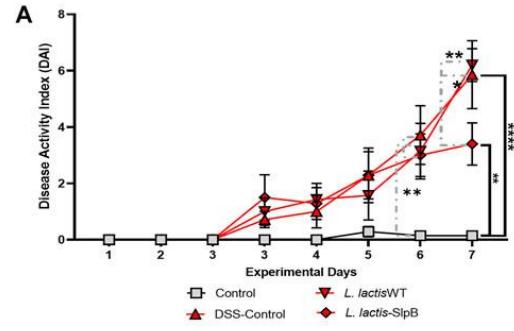


> Impact on anti-inflammatory effect



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Impact on anti-inflammatory effect

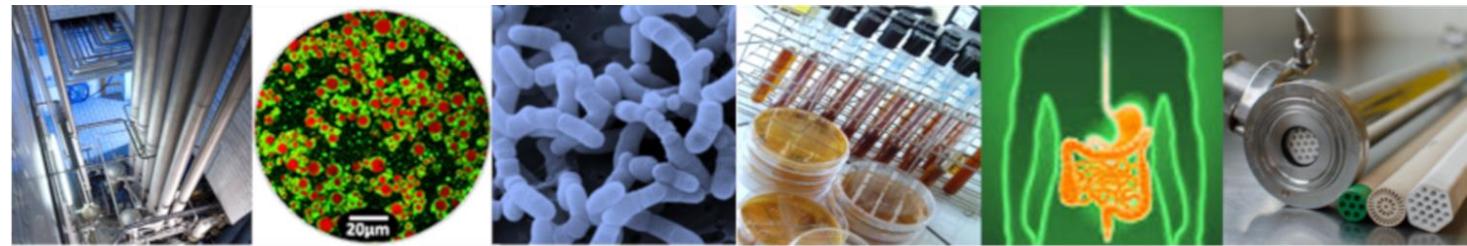


SlpB expression provides *L. lactis* with enhanced anti-inflammatory properties

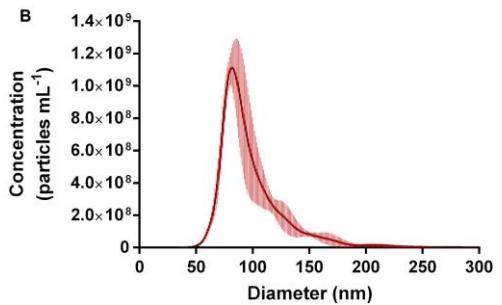
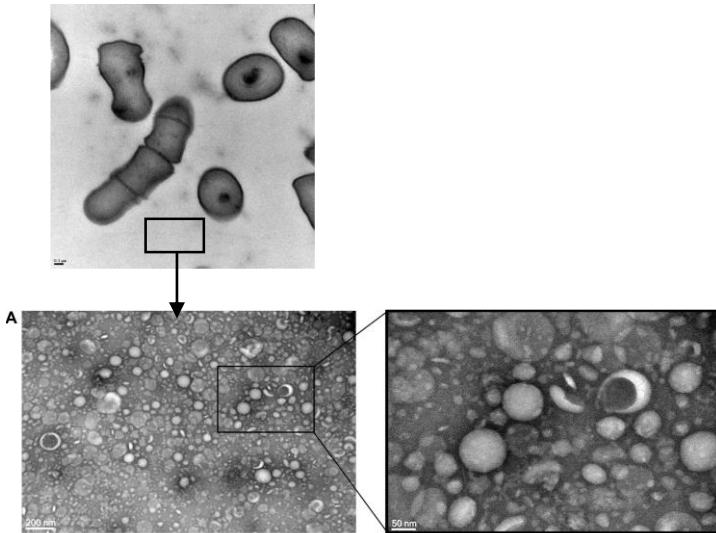


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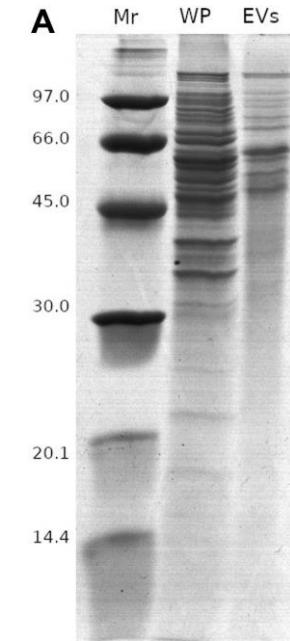
> What about Evs ?



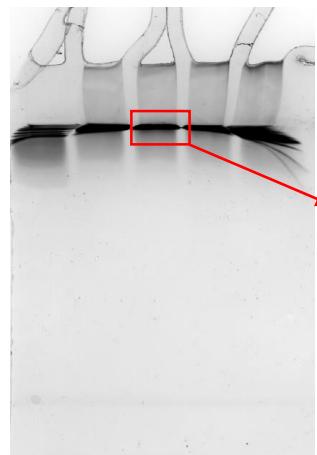
> Propionibacteria produce EVs



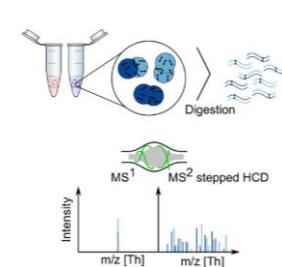
→ SDS solubilisation → SDS PAGE



Barely run SDS PAGE



In-gel trypsin digestion



Nano-LC-MS/MS
Q Exactive



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Propionibacteria immunomodulation
And EVs



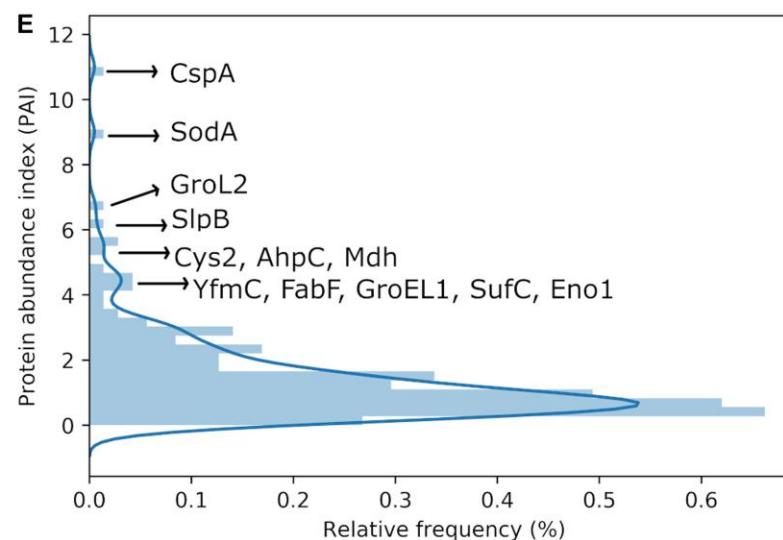
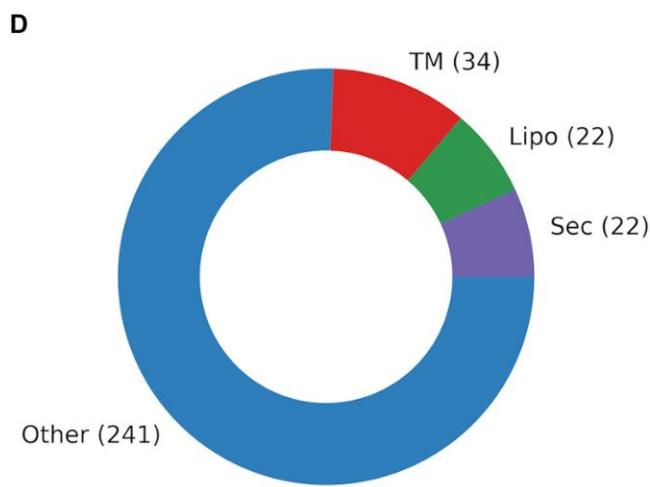
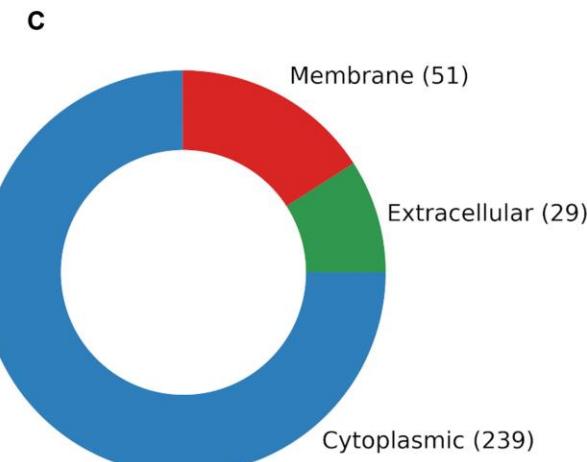
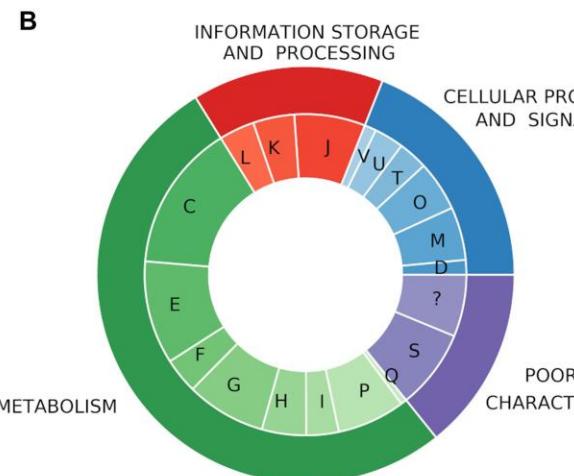
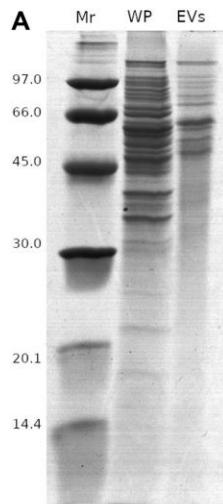
Vinicius Rodovalho
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Eric Guédon, INRAE, STLO



> Propionibacteria EVs contain (surface) proteins





➤ 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?”

