The dairy bacterium Propionibacterium freudenreichii against colitis and mucositis: a key role of the surface layer protein SlpB

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The dairy probiotic bacterium *Propionibacterium freudenreichii* against colitis and mucositis: a key role of the surface layer protein SlpB

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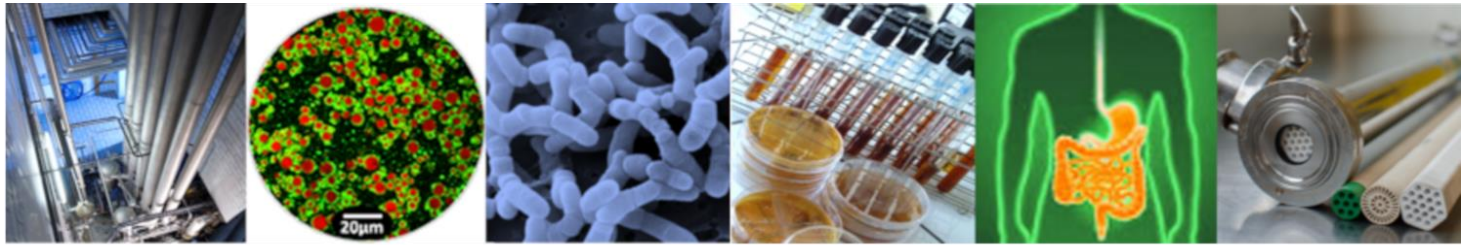
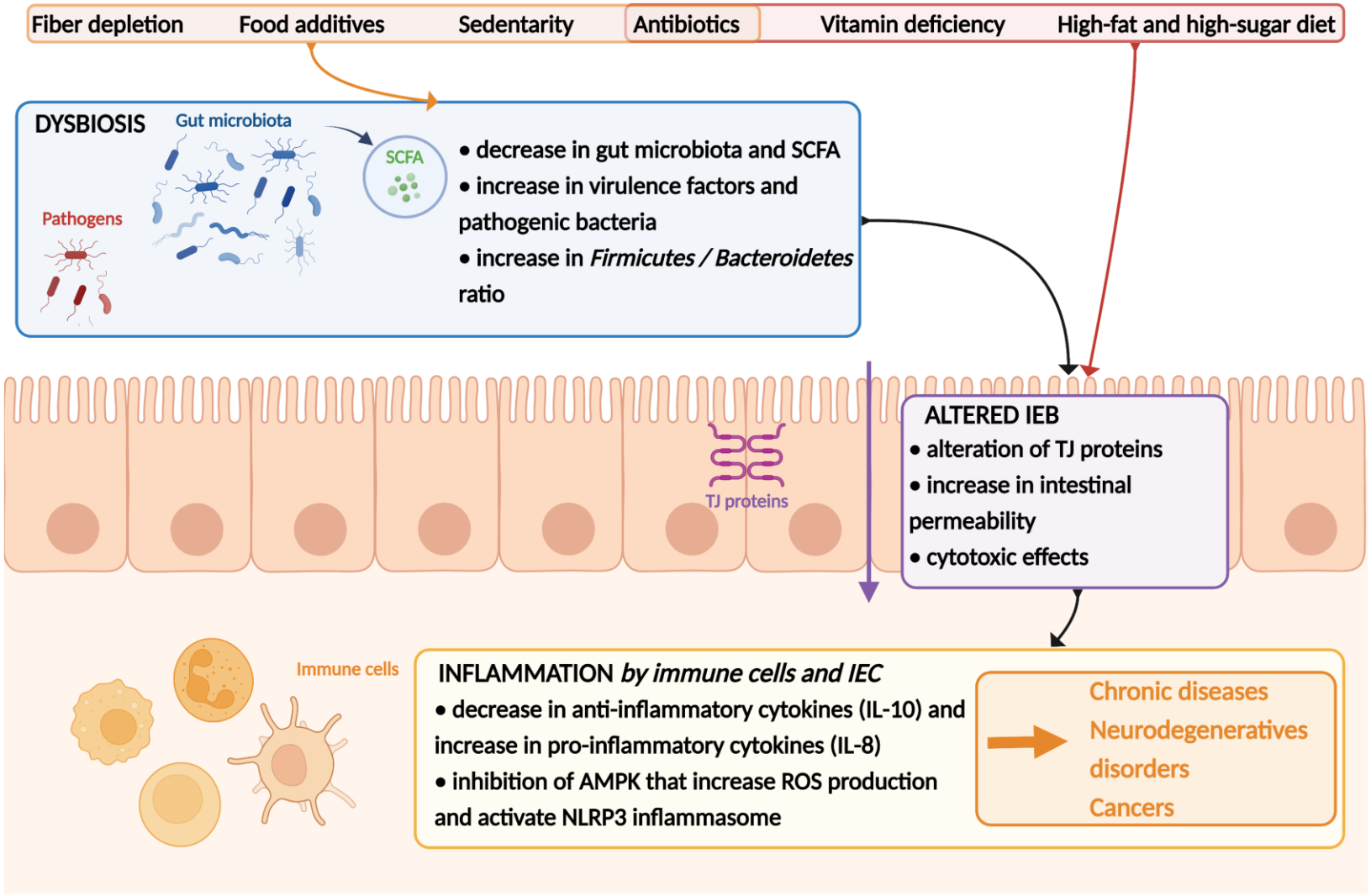
Intestinal epithelial barrier: endangered…



… By modern lifestyle



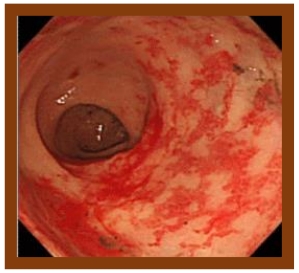
Examples of digestive inflammatory diseases

Colitis

- GIT Chronic inflammation

Ulcerative Colitis

Mucositis



GI Mucositis

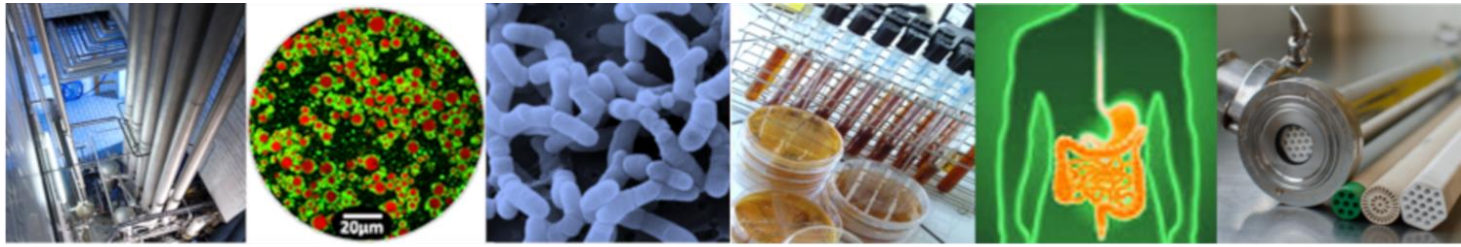
Oral mucositis

5-Fluoracil (5-FU)

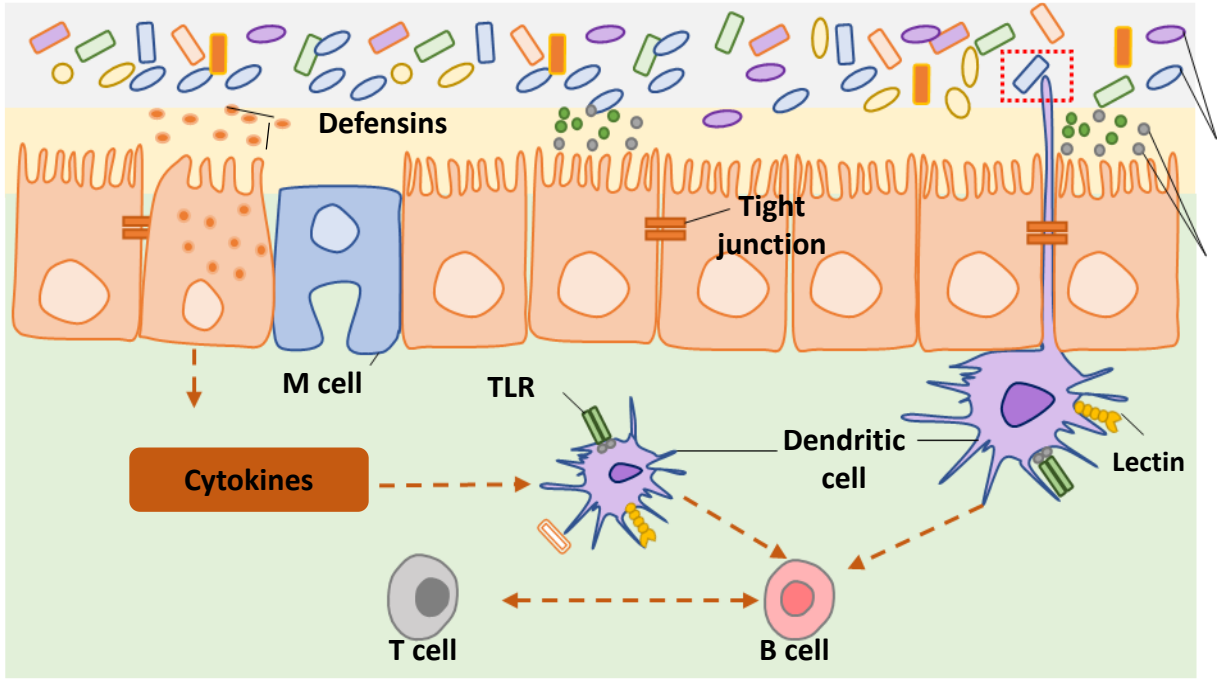
- Head, neck and colon cancer.

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

A key role of resident and ingested microorganisms…

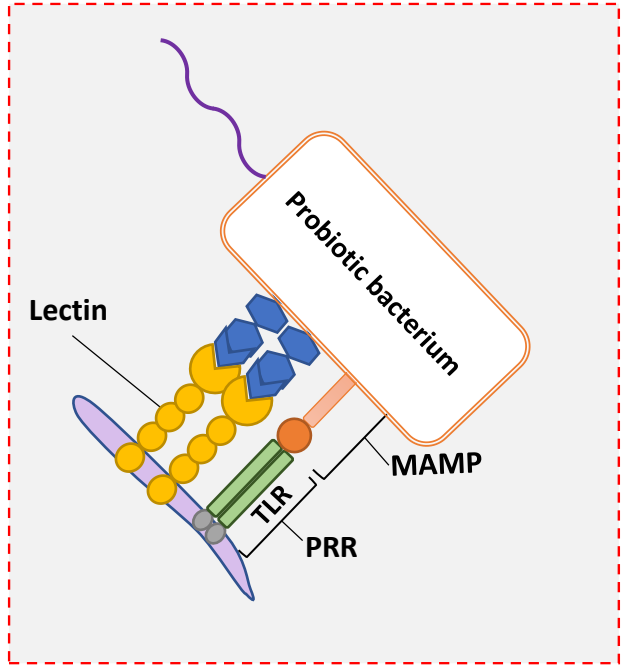


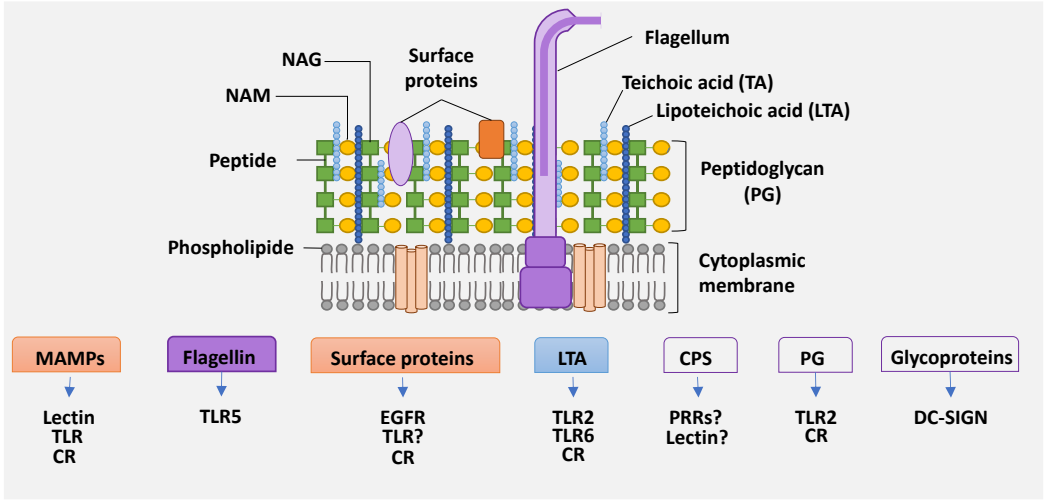
… Modulating the immune response



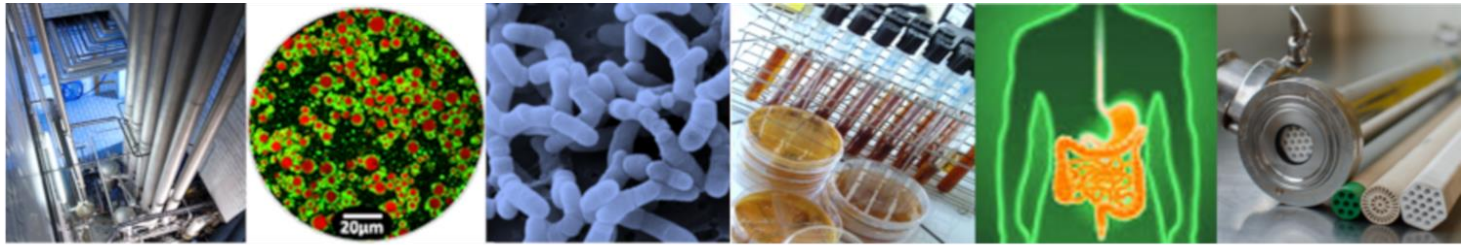
Intestinal microbiota

Antimicrobial peptides

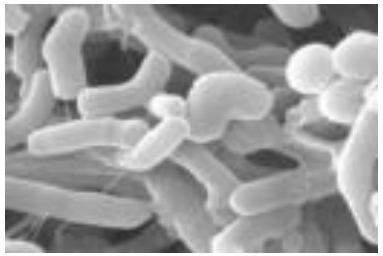




Introducing *Propionibacterium freudenreichii*…

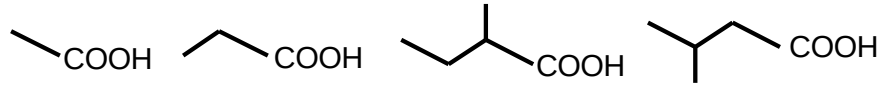


… A sort of Swiss army knife



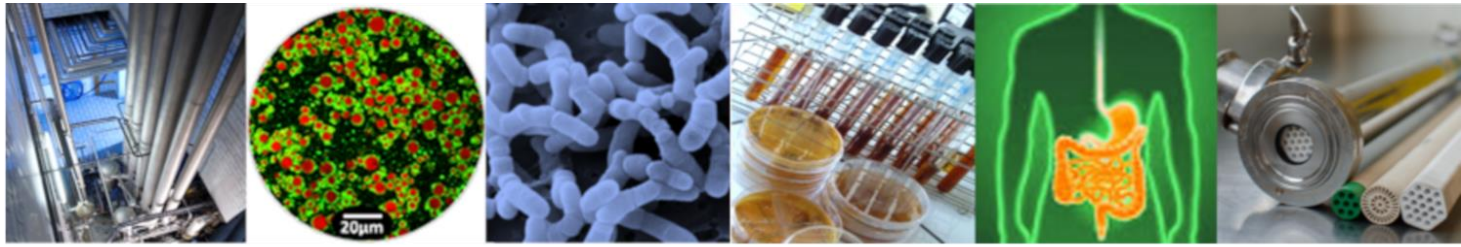
Propionibacterium freudenreichii

- ➔ Actinobacterium 
- ➔ Vitamin producer B9, B12 
- ➔ Probiotic 
- ➔ Food bio-preservative 
- ➔ Ripening starter 
- ➔ Short chain fatty acids



Short Chain Fatty Acids (SCFA) Known effect on health

Immunomodulatory properties

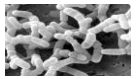


… *In vitro* and *in vivo*

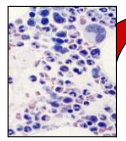
benoit.foligne@univ-lille2.fr

bruno.pot1@telenet.be

♦ *In vitro*



+



PBMCs

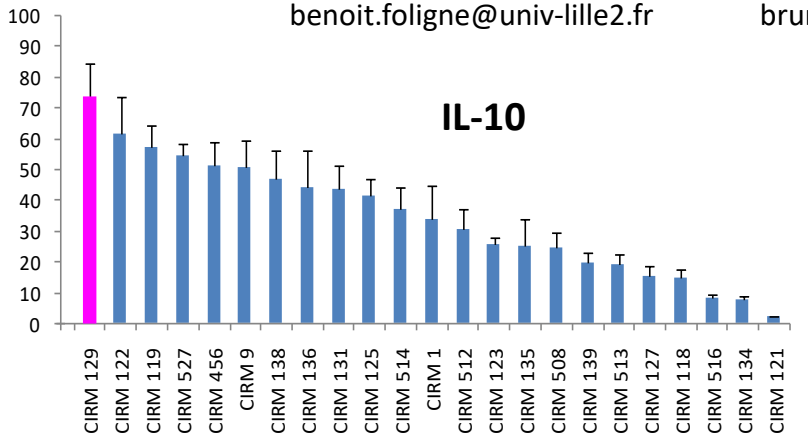
24 h

IL-10

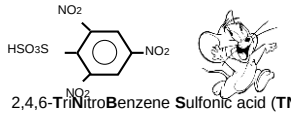
IFNγ

IL-12

TNFα

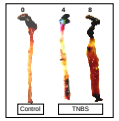


♦ *In vivo*

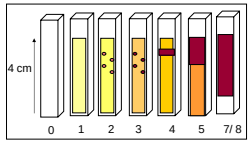


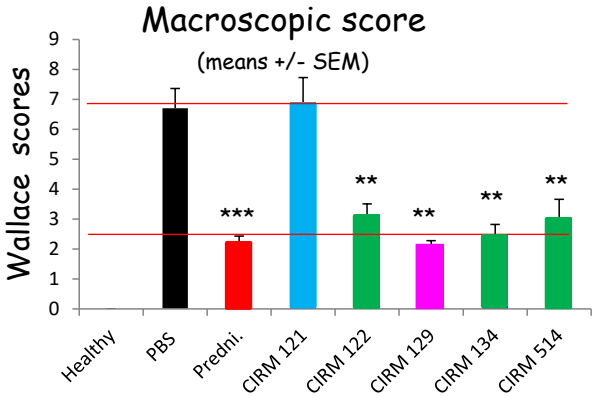
2,4,6-TriNitroBenzene Sulfonic acid (TNBS)

Acute colitis



Macroscopic score (Wallace)

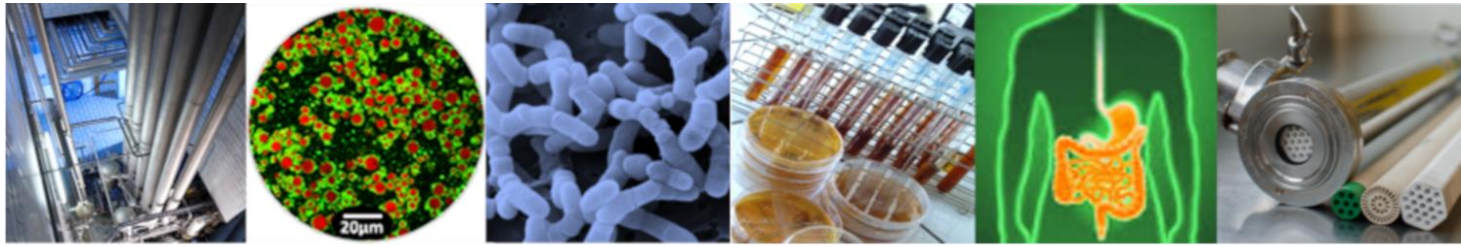




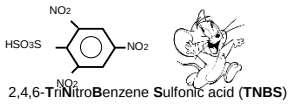
SURFING
Starter Surface
against Inflammation of the Gut

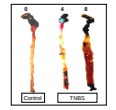
CIIL
CENTER FOR INFECTION
& IMMUNITY OF LILLE

The healing effect of *Propionibacterium freudenreichii*



In TNBS-induced colitis





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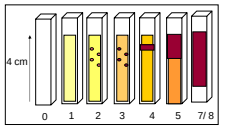
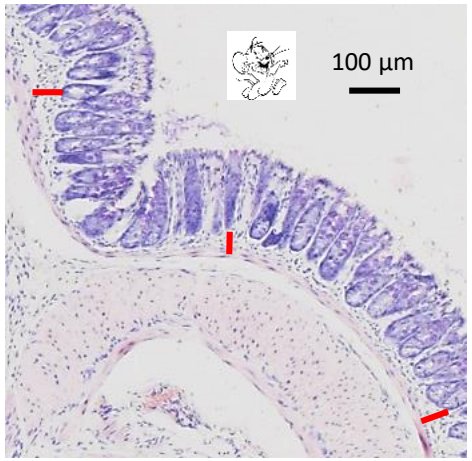
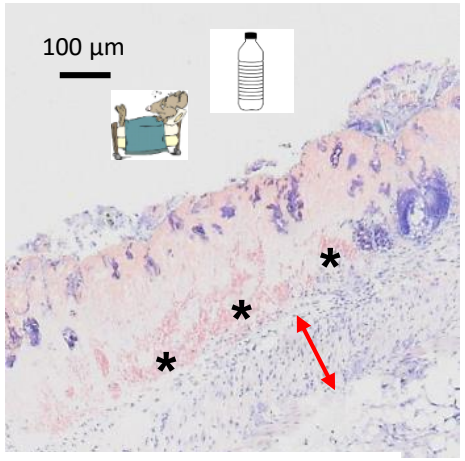
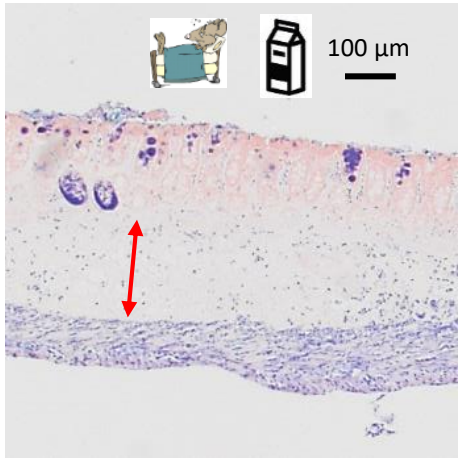
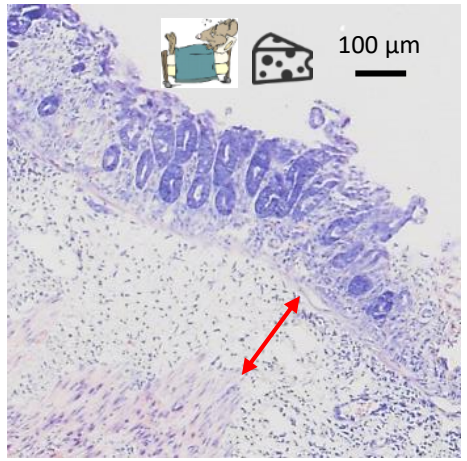


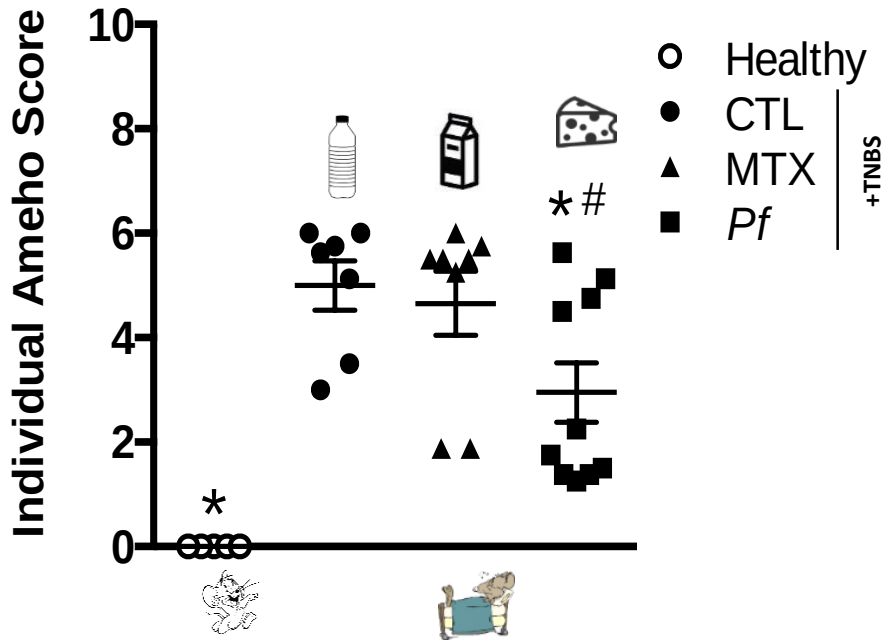
A model cheese











CIIL
CENTER FOR INFECTION & IMMUNITY OF LILLE

In DSS-induced colitis

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

C57BL6
8th week age

1st Day

5th Day

12th Day

Intragastric gavage

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

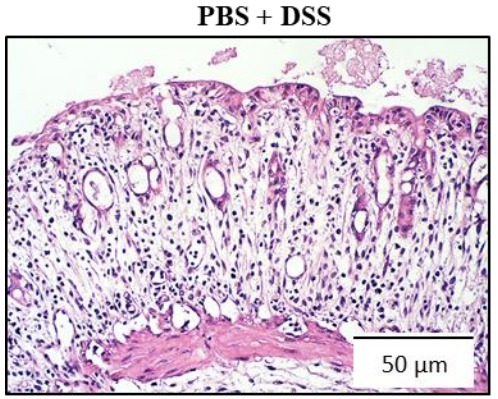
3% (w/v) **DSS solution**
(36–50 25 kDa)

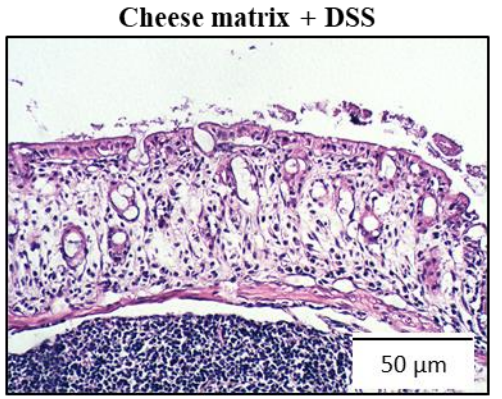
Colitis induction

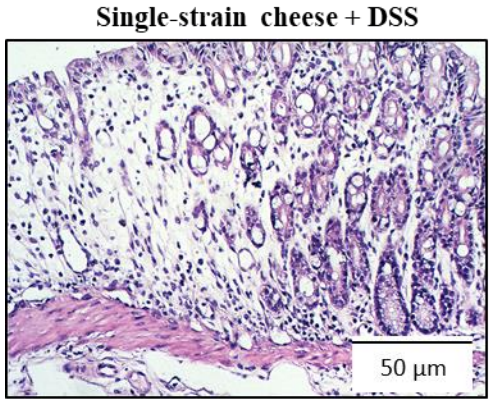
Beginning

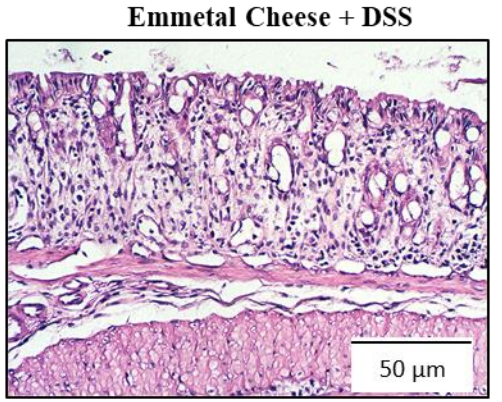
Euthanasia

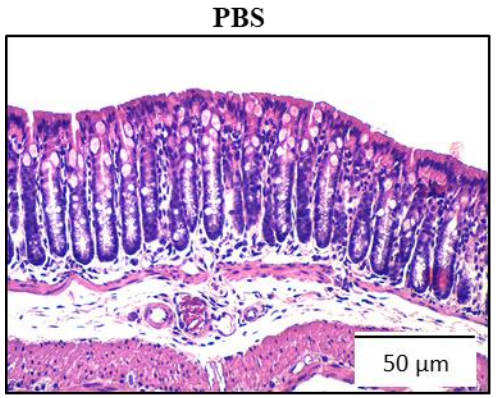
Vasco Azevedo

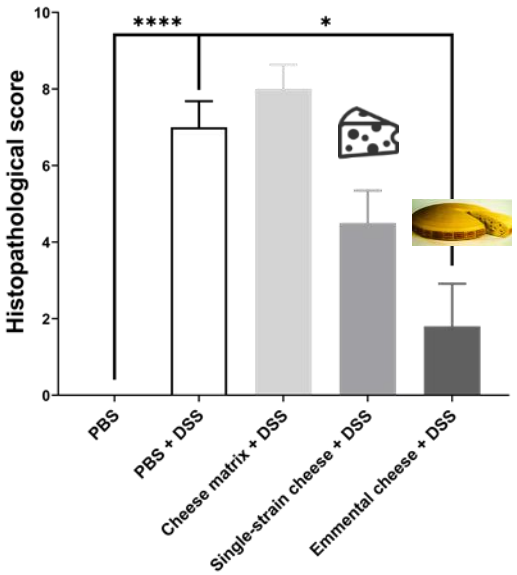












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8th week age

1st Day — **5th Day** — **12th Day**

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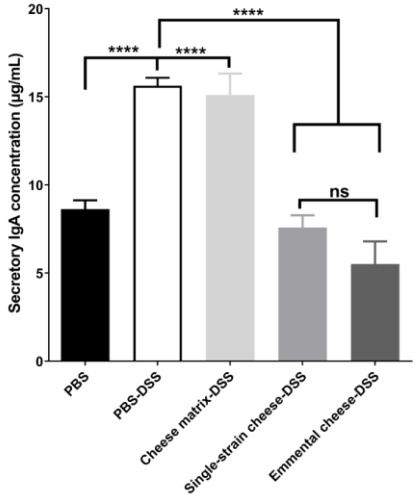
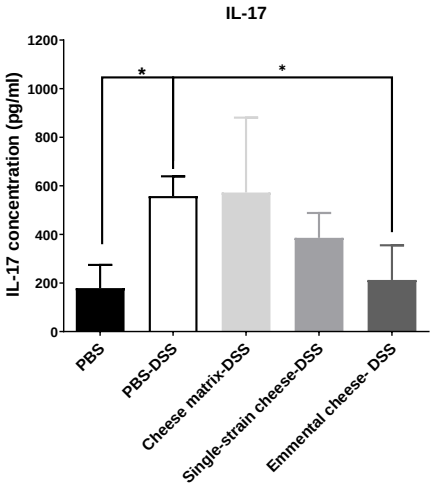
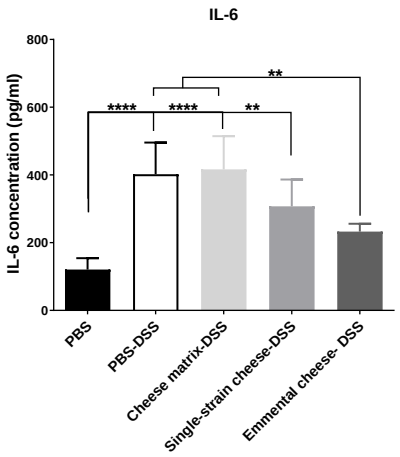
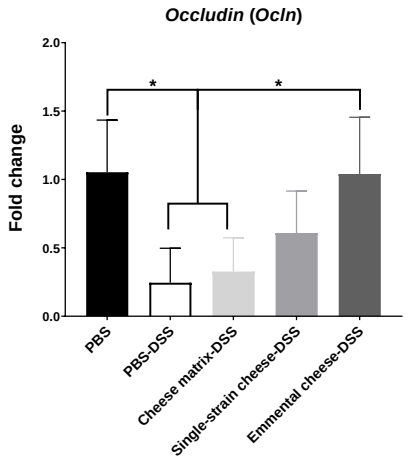
Colitis induction

Beginning — **Euthanasia**

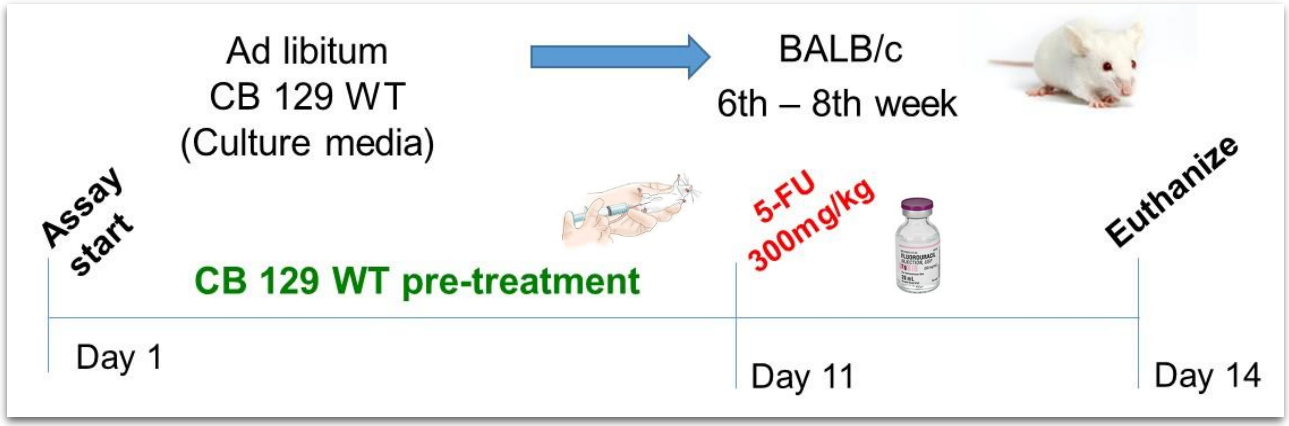




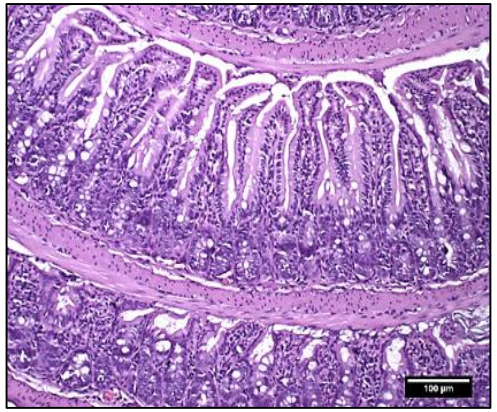




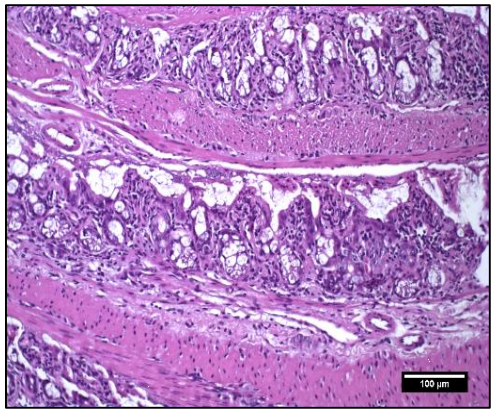
In 5FU-induced mucositis



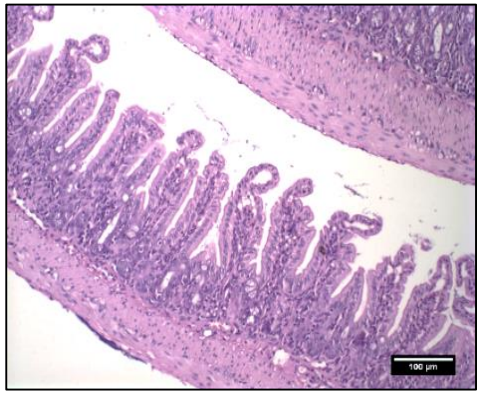
Representative images from mucosal histopathology (H&E)



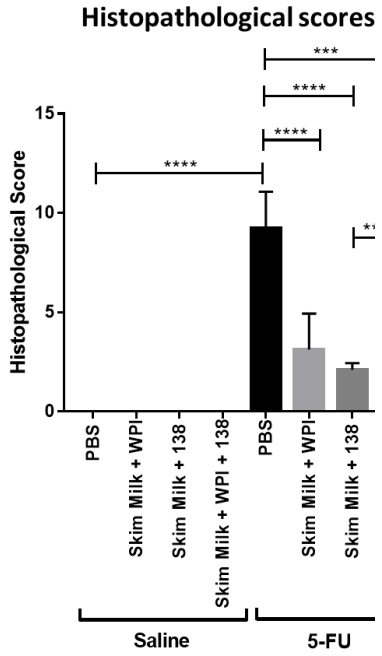
Healthy Ileum



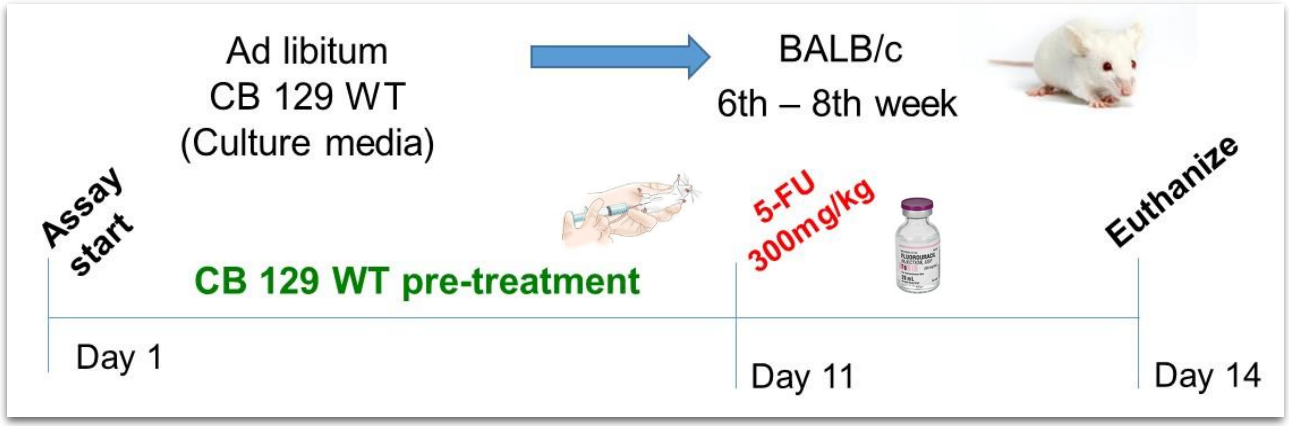
PBS + 5-FU



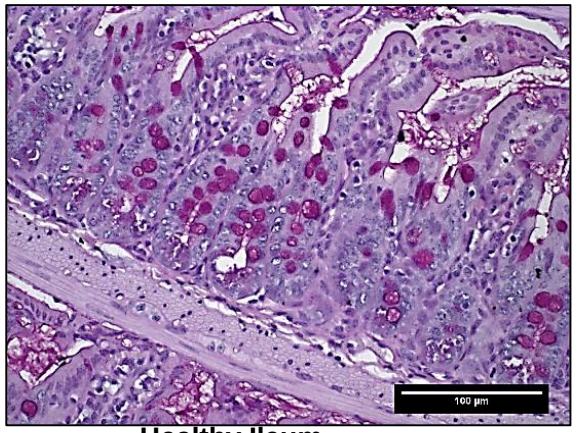
Skim milk + propio



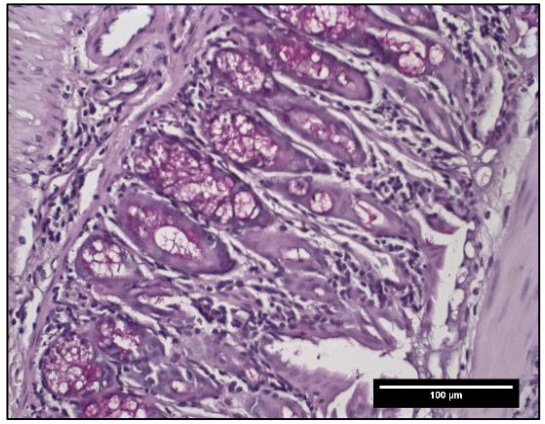
In 5FU-induced mucositis



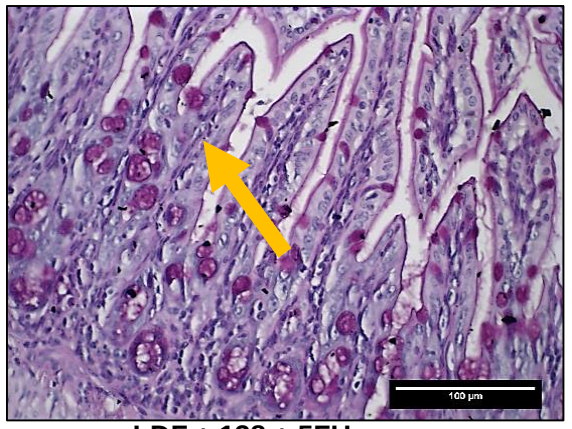
Representative images of stained goblet cells (PAS)



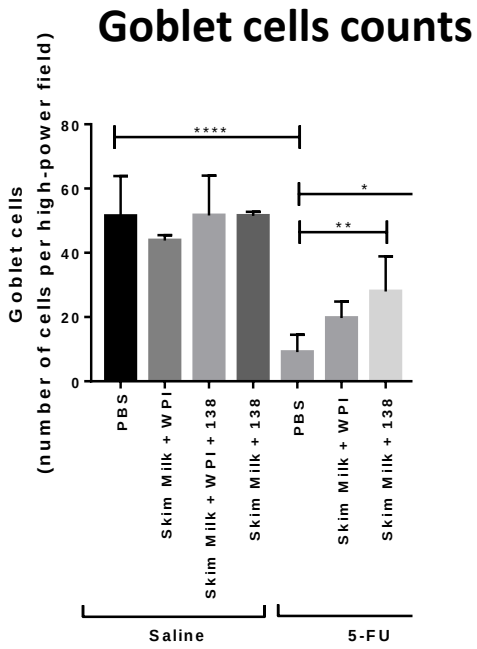
Healthy Ileum



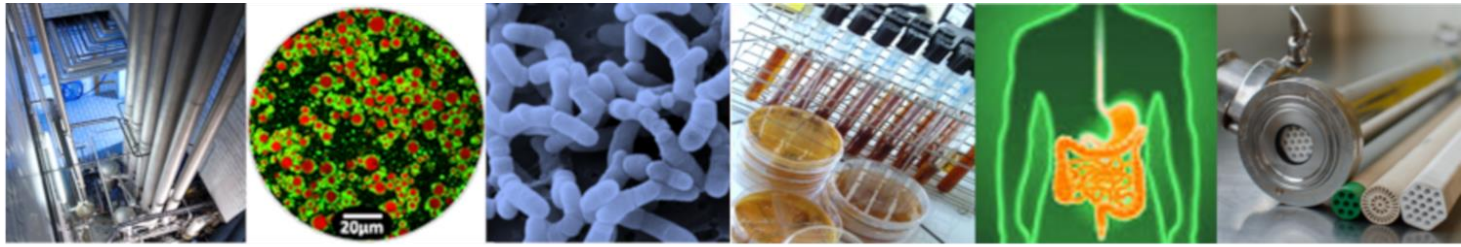
PBS + 5FU



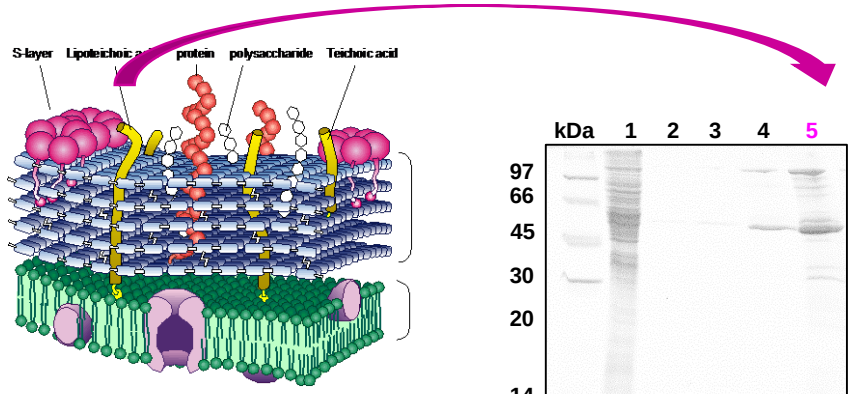
LDF + 138 + 5FU



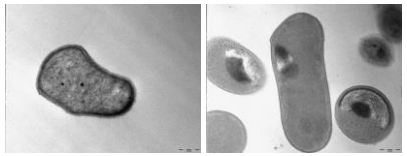
How does it work?



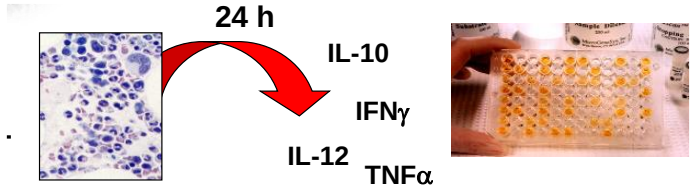
If we remove surface proteins: PBMC assay



← SlpB



Untreated Guanidine treated



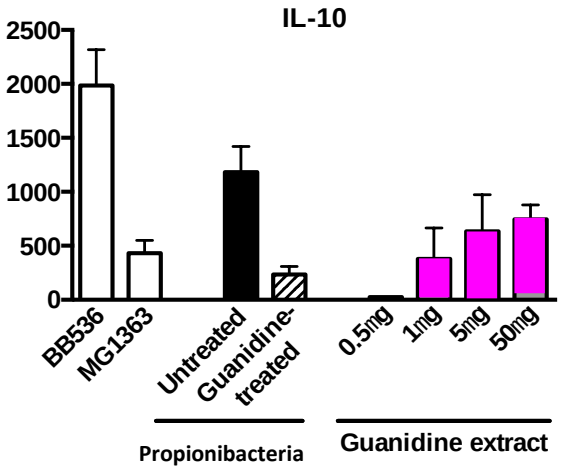


Table 1 – *Propionibacterium freudenreichii* proteins identified by nano-LC-MS/MS after guanidine hydrochl (shaving column) or *in-situ* fluorescence labeling[a] (CyDye column).

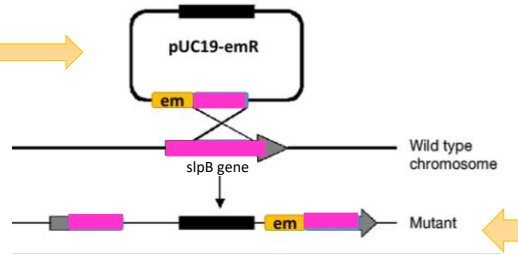
Locus Tag	Description	Gene	Function
PFCIRM129_12235	Internalin A	inlA	Miscellaneous
PFCIRM129_05460	Surface protein with SLH domain	slpE	Cell wall
FCIRM129_09350	Surface layer protein A	slpA	Cell wall
PFCIRM129_00700	Surface layer protein B	slpB	Cell wall
PFCIRM129_11445	Large surface protein A	lspA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

Stripped propionibacteria loose immunomodulatory properties

But extracted proteins induce IL-10

If we mutate Surface Layer Protein genes: PBMC assay

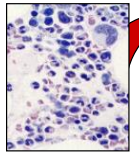
Integrative plasmids (suicide vectors)



Gene disruption by single cross-over plasmid insertion

♦ *In vitro*

+



24 h

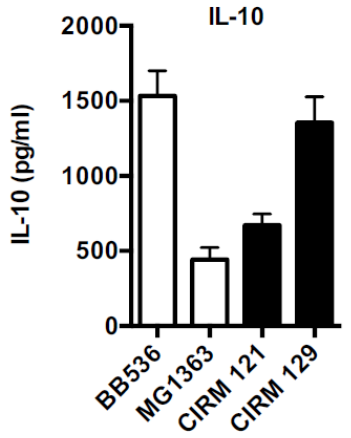
IL-10

IFNγ

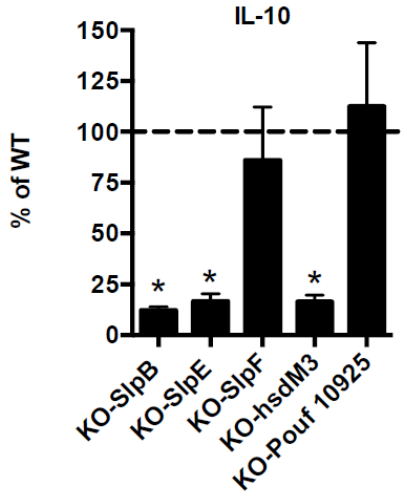
IL-12

TNFα

A

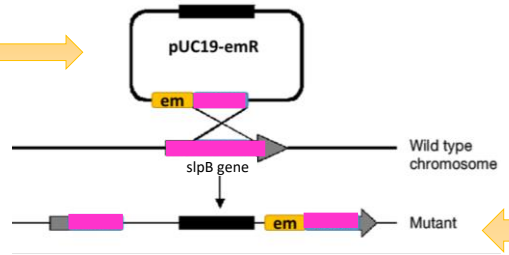


B

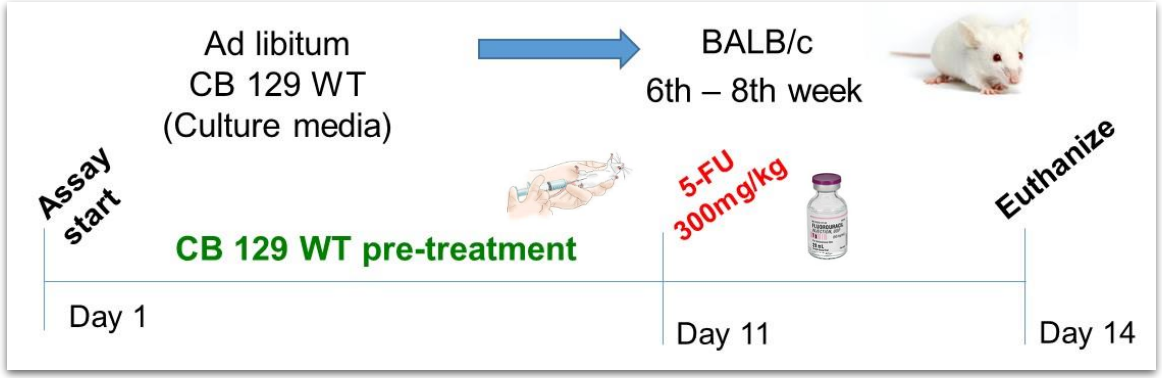


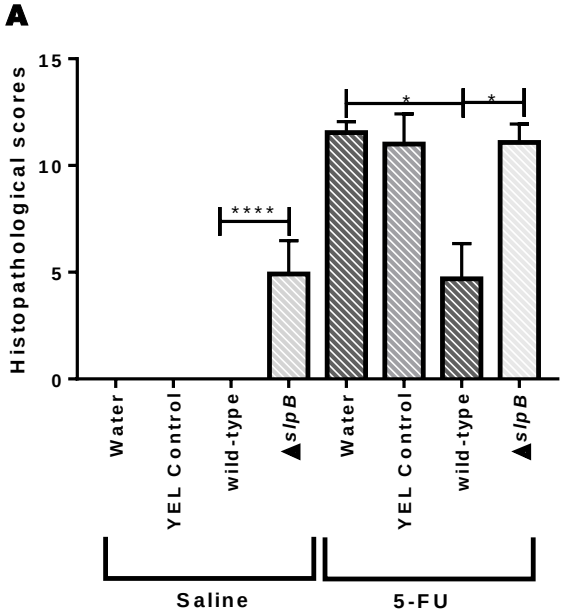
If we mutate Surface Layer Protein SlpB gene: mucositis challenge

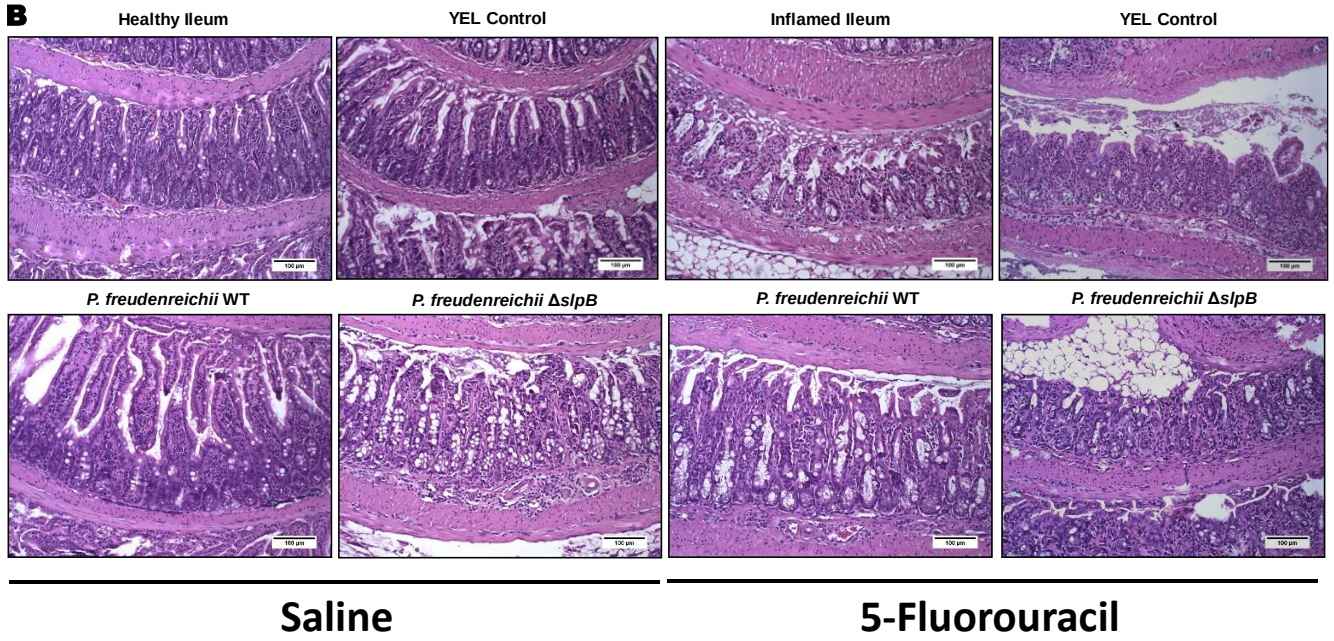
Integrative plasmids (suicide vectors)



Gene disruption by single cross-over plasmid insertion







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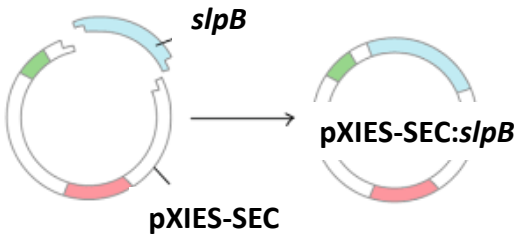
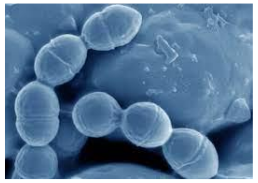
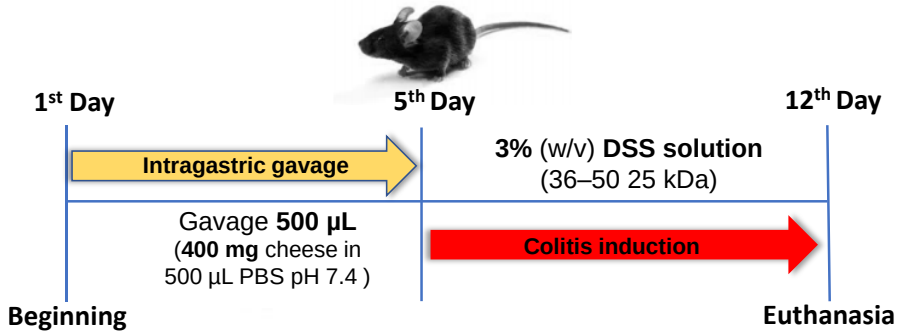
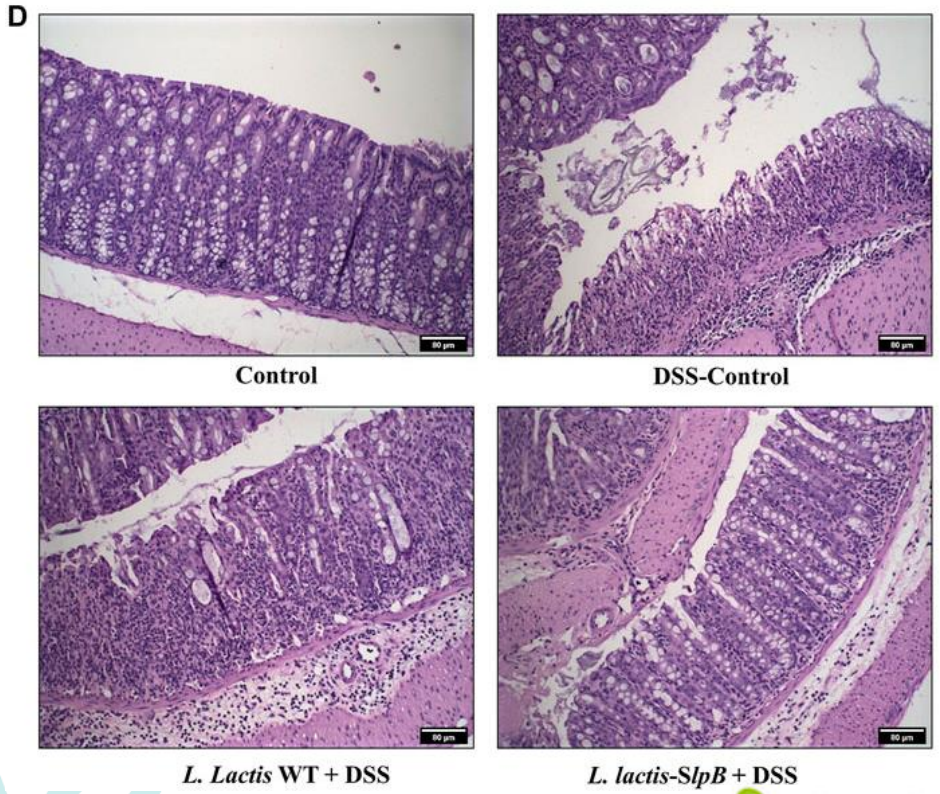
If we express SlpB in *Lactococcus lactis*: colitis challenge

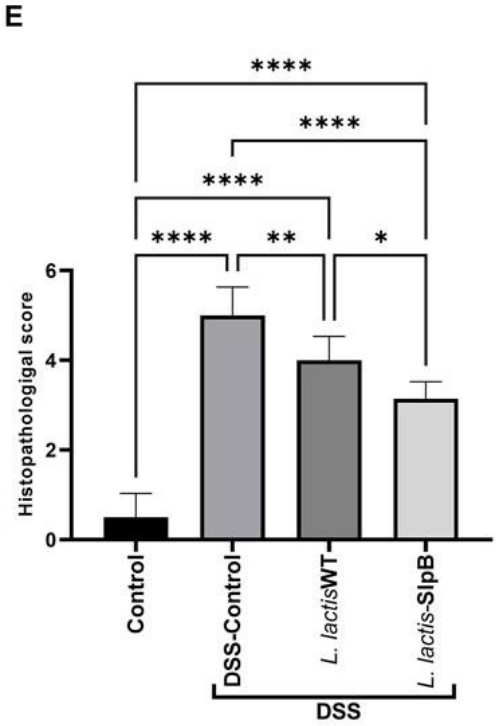




Lactococcus lactis







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

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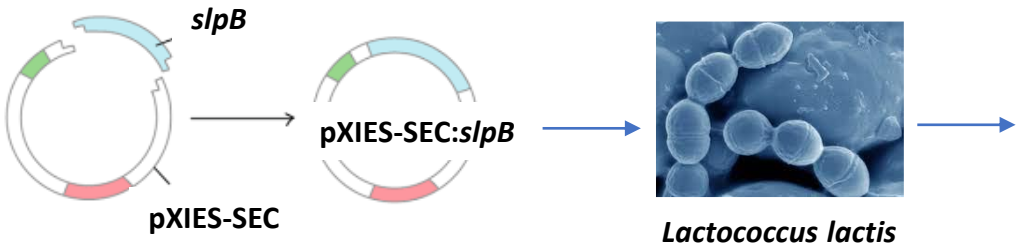
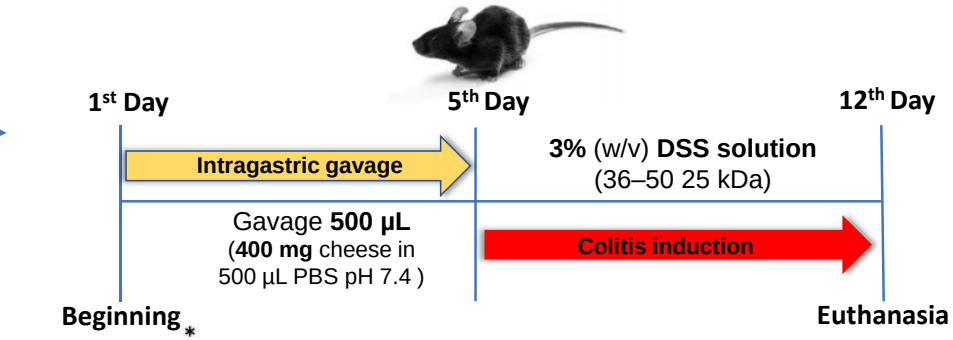
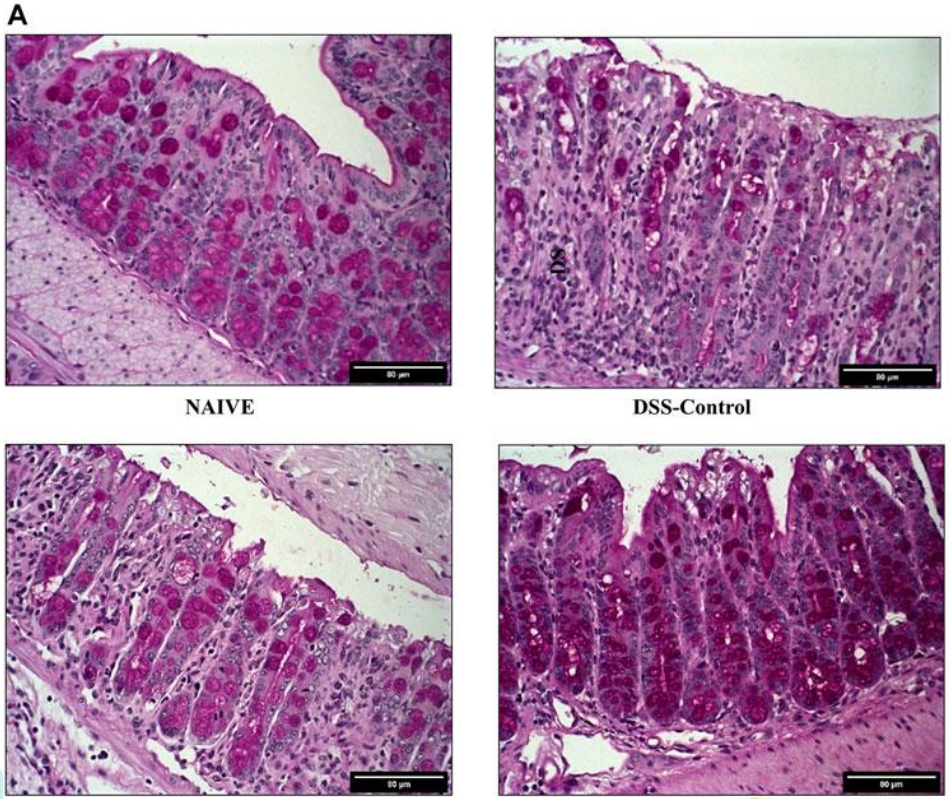
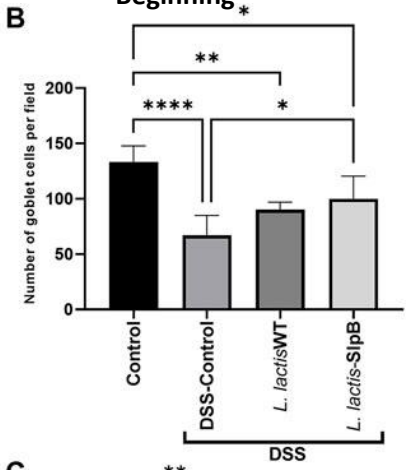
If we express SlpB in *Lactococcus lactis*: colitis

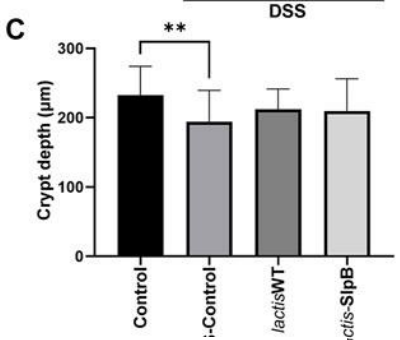


Lactococcus lactis









SlpB expression protects from mucus and goblet cells depletion

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

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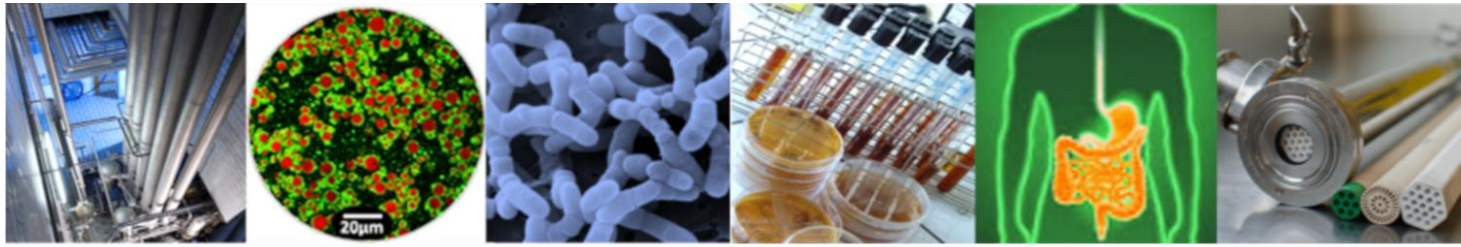
Take-home messages

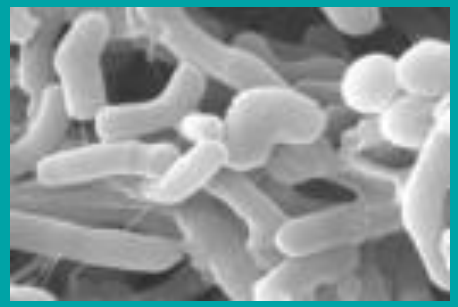


- **Propionibacteria: strain-dependent immunomodulatory properties**
- **Anti-inflammatory effect of propionibacteria-containing cheese in different models of inflammatory diseases**
- **Surface layer protein SlpB plays a key role in this interaction**
- **This can be transferred to another bacterium via heterologous expression**

I ♥ Γραβιέρα Κρήτης

INRAE

Thank you for your attention



I ♥ Emmental

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