



Cheese matrix protects the immunomodulatory surface protein SlpB of *Propionibacterium freudenreichii* during *in vitro* digestion

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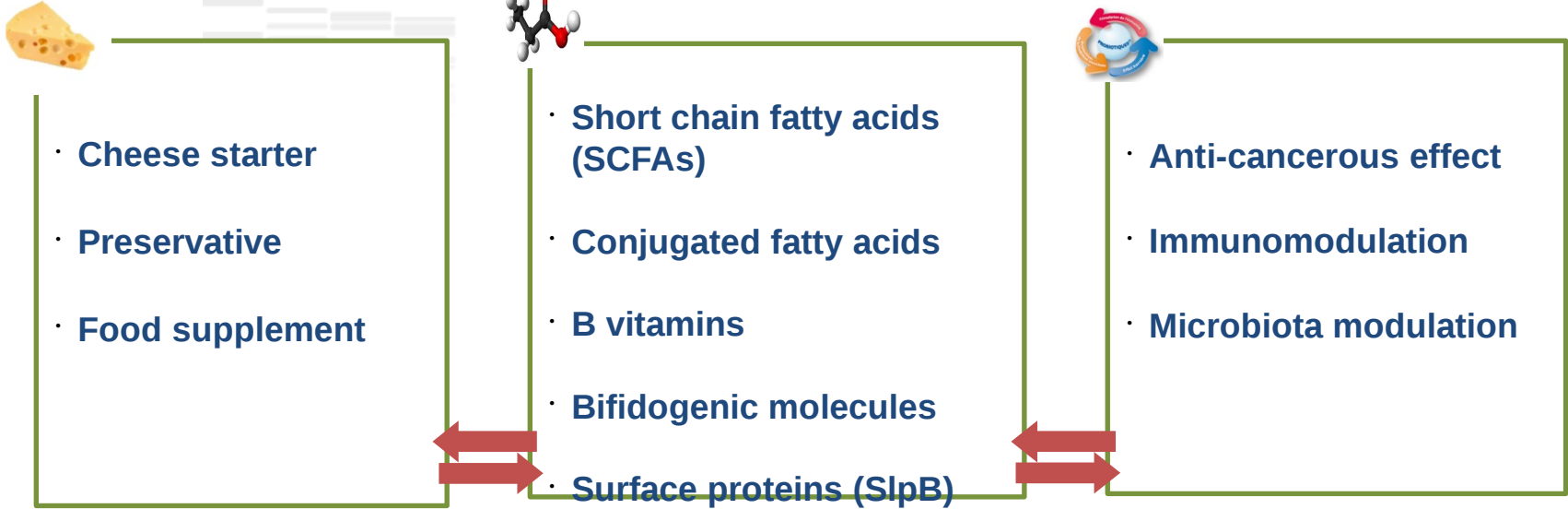
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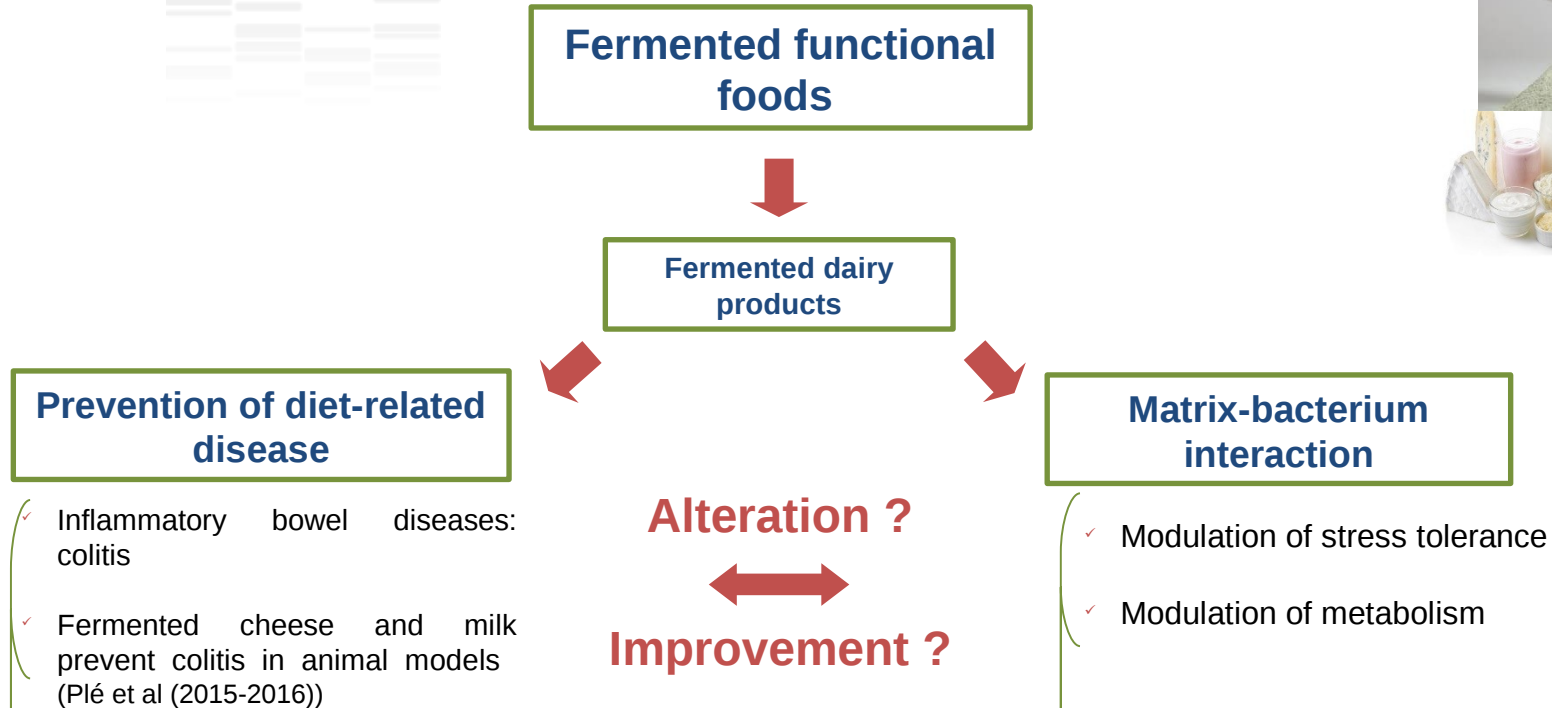
Propionibacterium freudenreichii



Strain-dependent properties

A « 2 in 1 » Bacterium

P. freudenreichii: functional foods application



Is *P. freudenreichii* immunomodulatory effect matrix-dependent?



Production of
immunomodulator
y metabolites



Expression of
surface proteins



Protection of
surface proteins
against digestive
proteolysis



Tolerance to
digestive stresses

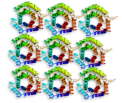


Variation of immunomodulatory property

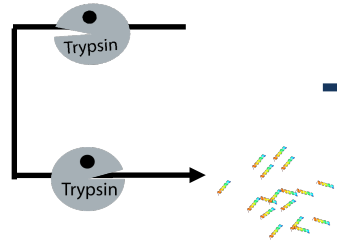
Does the proteolysis abolish the anti-inflammatory effect of Slps ?

Experimental strategy

nreichii



ides



HT-29 cells stimulation



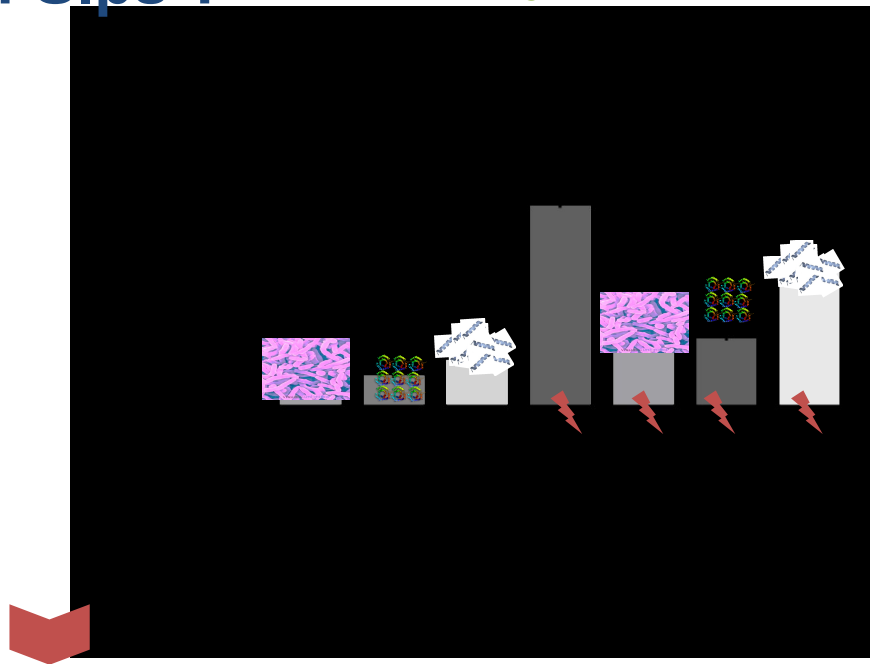
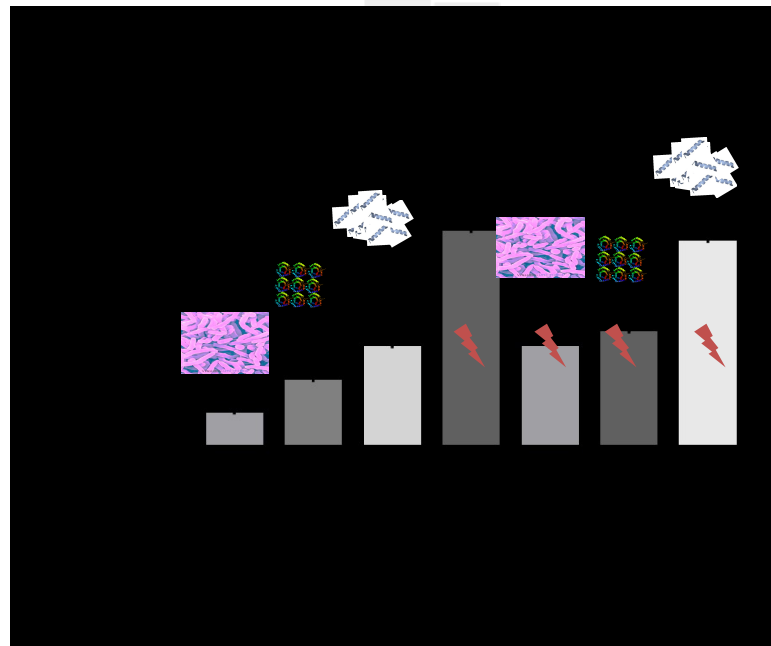
RT-PCR of cytokines



+/- LPS (*E. coli*)



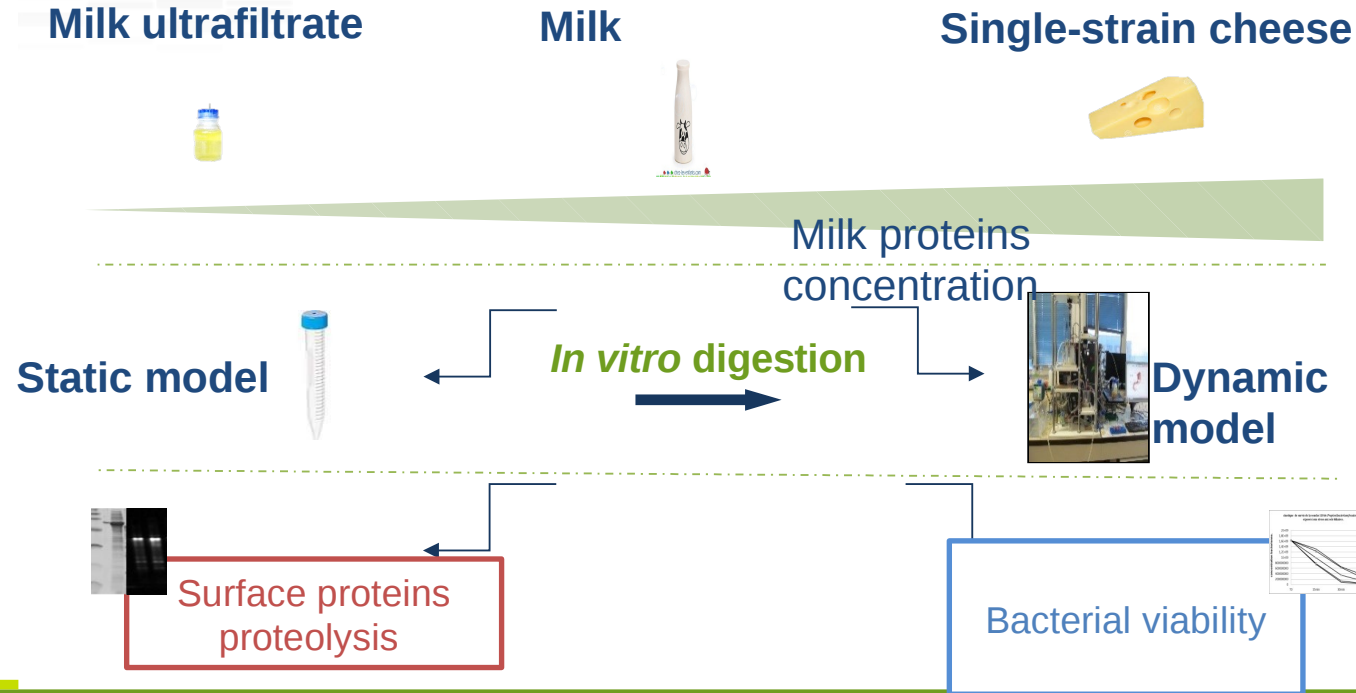
Does the proteolysis affect the anti-inflammatory effect of Slps ?



Slps proteolysis abolished its immunomodulatory properties

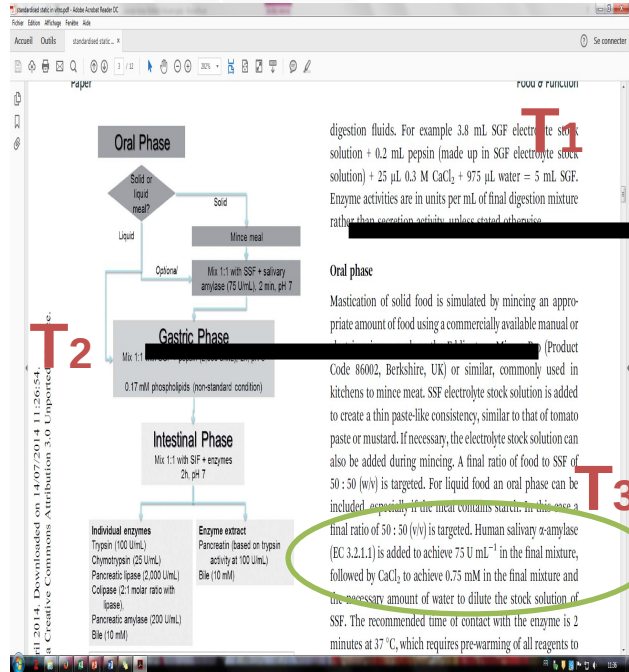
In vitro assessment of *P. freudenreichii* delivery

Experimental strategy

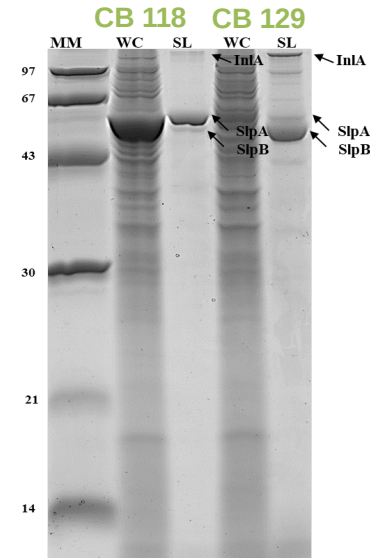


In vitro Static digestion model

- Standard experiment protocol (Minekus, et al. (2014))
 - Relatively easy - absence of kinetics



- First screening:
 - Two strains were tested: *P. freudenreichii* CIRM-BIA 118 & 129
 - Bacterial pellet extraction protocol from cheese was perfected -> For Western blot analyses

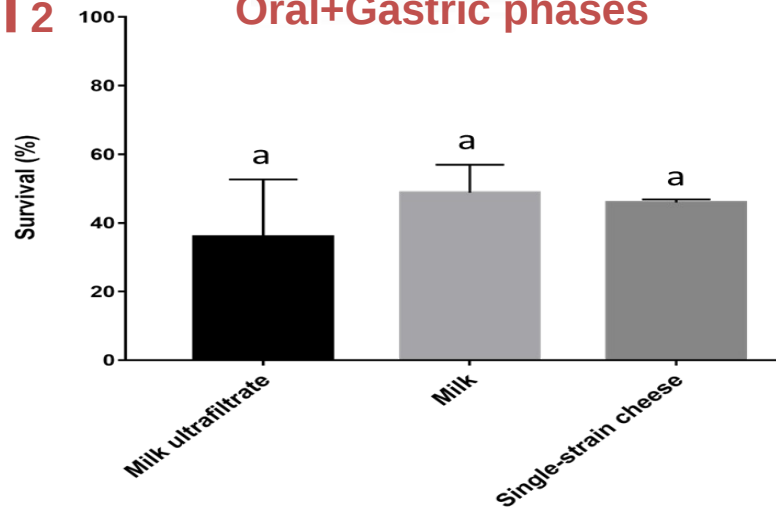


Cheese matrix enhances bacterial viability during *in vitro* static digestion

P. freudenreichii CIRM-BIA
118

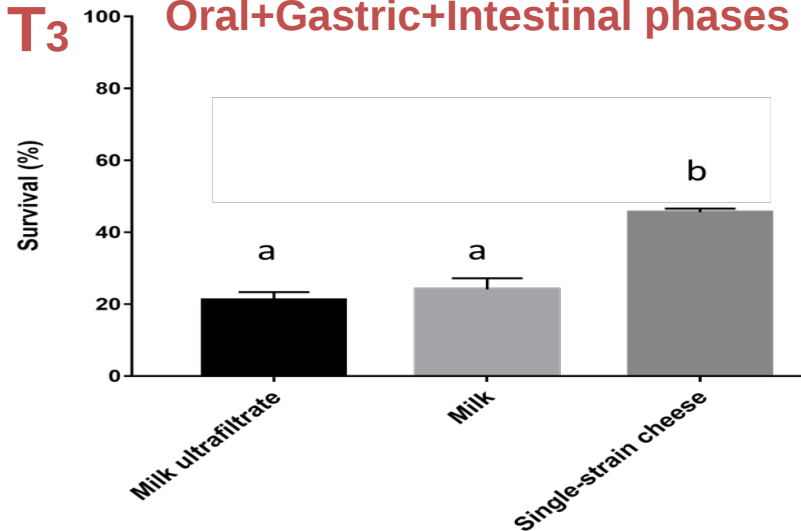
T₂

Oral+Gastric phases



T₃

Oral+Gastric+Intestinal phases

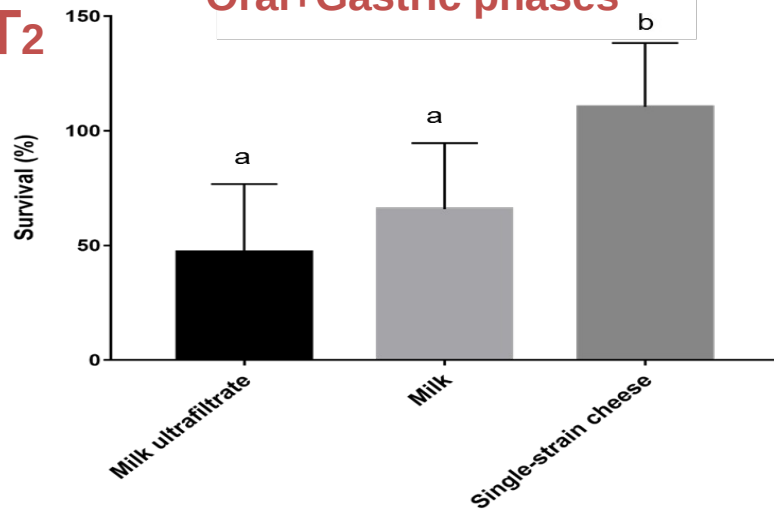


Cheese matrix enhances bacterial viability during *in vitro* static digestion

P. freudenreichii CIRM-BIA

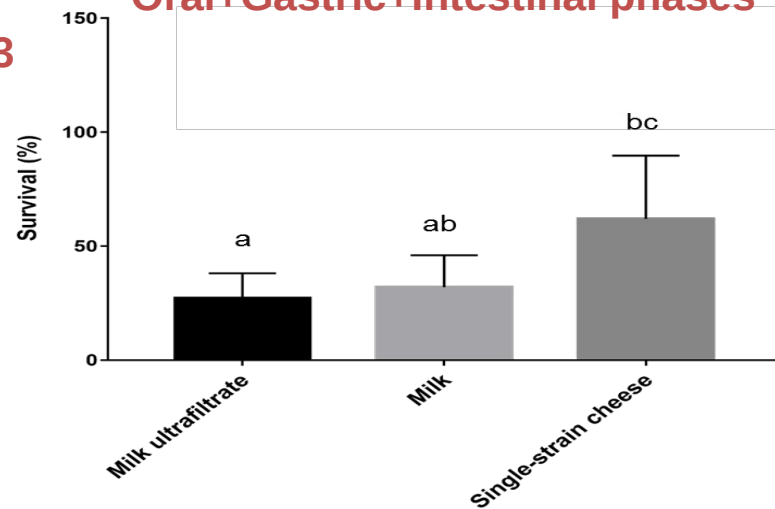
Oral+Gastric phases

T₂



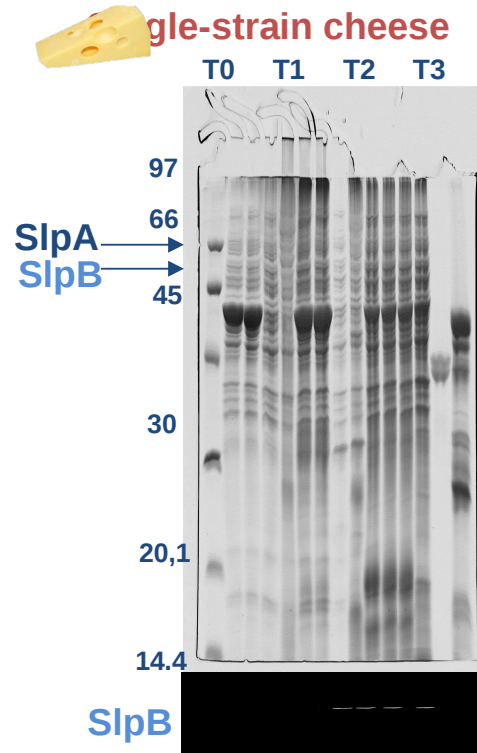
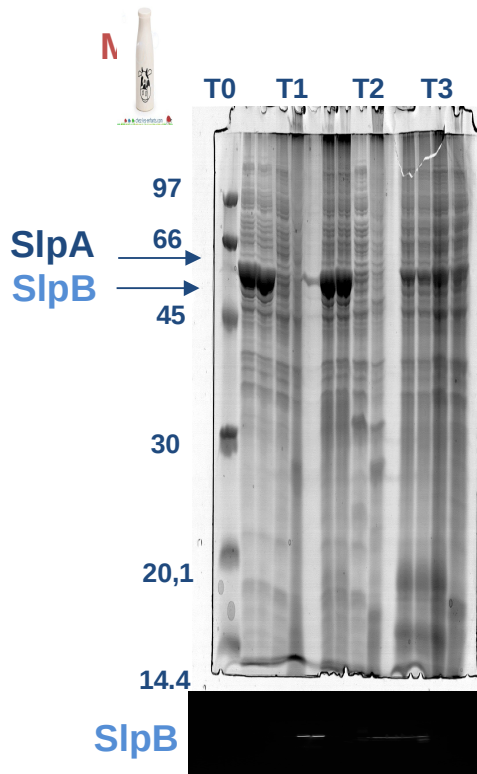
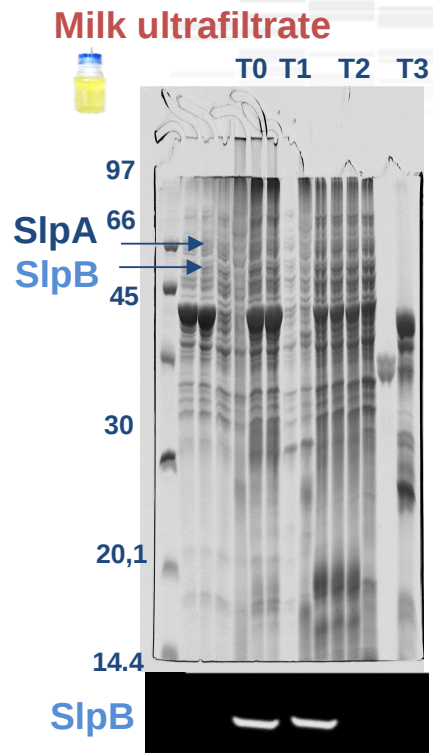
Oral+Gastric+Intestinal phases

T₃



Cheese matrix protects SlpB from digestive proteolysis

P. freudenreichii CIRM-BIA 118

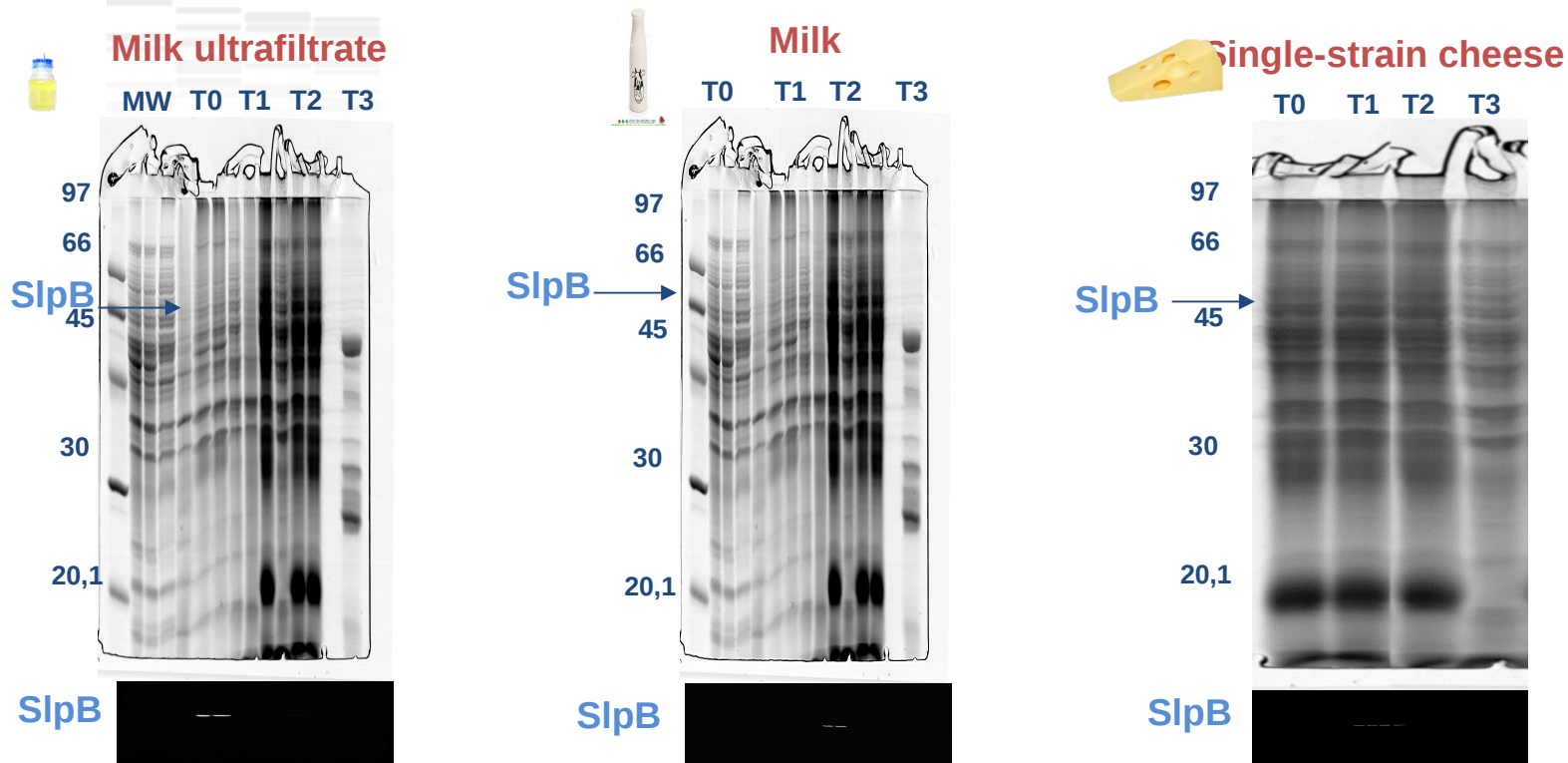


RESULTS

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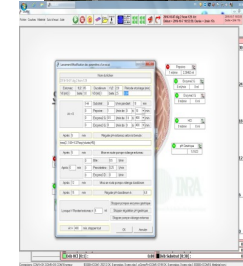
Cheese matrix protects SlpB from digestive proteolysis

P. freudenreichii CIRM-BIA 129

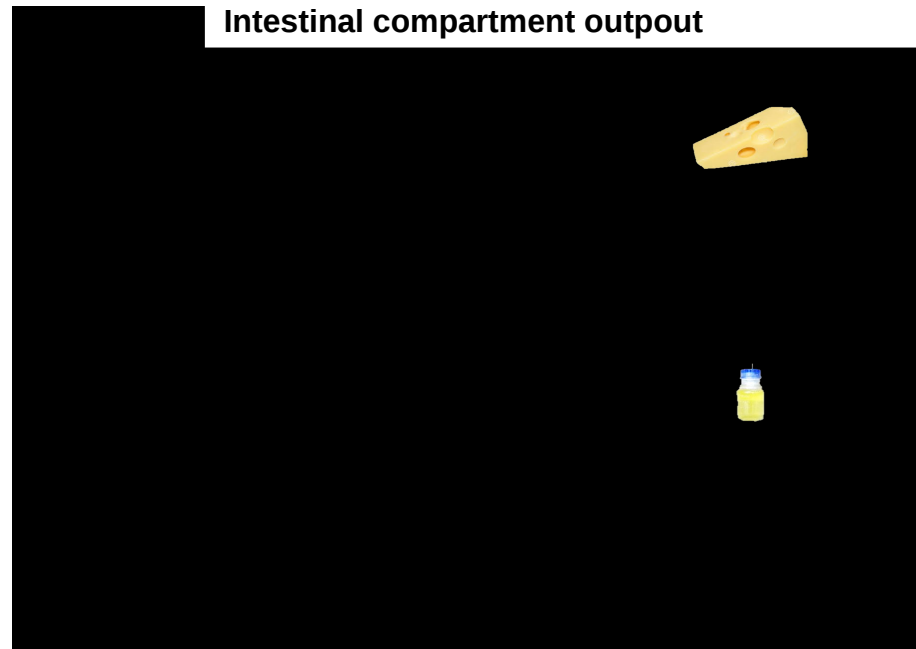


In vitro dynamic digestion model

- Dynamic digestion using Didgi® system:
 - ✓ Two compartments: gastric and intestinal
 - ✓ Two tested matrices : **cheese and milk ultrafiltrate**
 - ✓ One strain: *P. freudenreichii* CIRM-BIA 129
- Digestion was monitored by StoRM® software
- Same parameters were applied for both matrices: cheese parameters
- Sampling were performed:
 - ✓ **Gastric phase:** t=30, 60, 80, 120 et 180 minutes
 - ✓ **Intestinal phase:** t=120, 150, 195 et 240 minutes



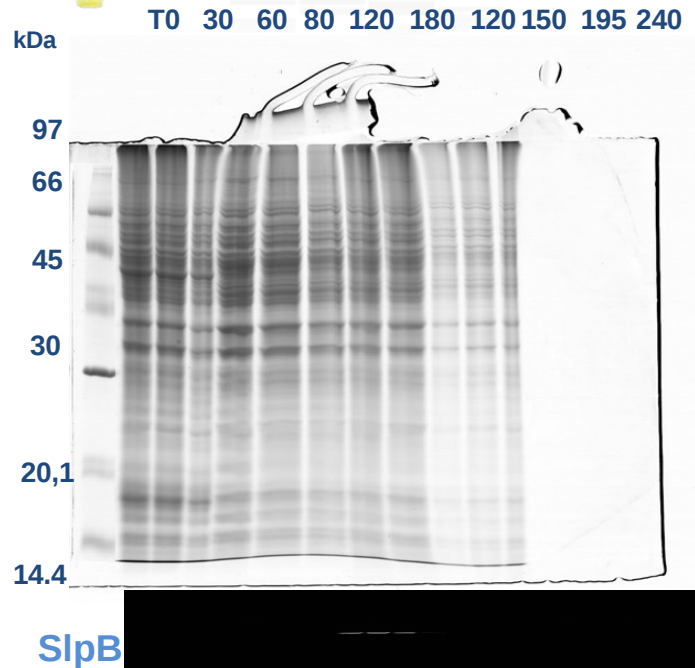
Cheese matrix enhances *P. freudenreichii* survival during *in vitro* dynamic digestion



Cheese matrix protects SlpB from digestive proteolysis

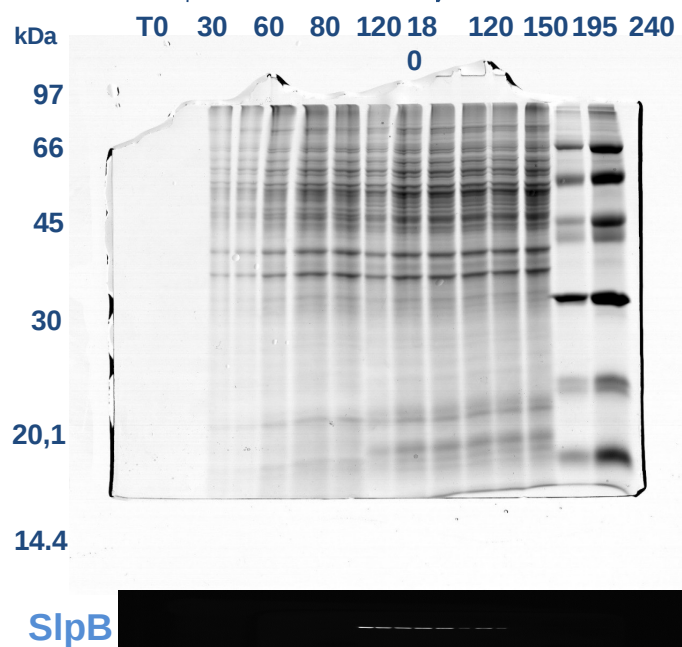
Milk ultrafiltrate

Gastric phase Intestinal phase



Single-strain cheese

Gastric phase Intestinal phase



Conclusions & Perspectives

- *In vitro* *P. freudenreichii* stress tolerance is matrix-dependent as was showed to be strain-dependent
- Cheese matrix enhances propionibacteria survival to *in vitro* digestive stresses
- Cheese matrix protects SlpB surface protein from *in vitro* digestive proteolysis
- *In vivo* *P. freudenreichii*' immunomodulatory properties **MAY be** matrix-dependent
- *In vivo* *P. freudenreichii*' immunomodulatory properties **MAY be** enhanced by cheese matrix
- *In vivo* experiment was carried out in healthy piglets -> confirmation in part of the *in vitro* results

MEP



**THANK
YOU FOR
YOUR ATTENTION**

Olivia Ménard



Floriane Gaucher



FL Rosa Do Carmo



Didier Dupont



Gwénaél Jan

