Do probiotic dairy starters adapt to vegetable milks?

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The stressing life of *Lactobacillus delbrueckii* subsp. *bulgaricus* in soy milk

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Differential Adaptation of *Propionibacterium freudenreichii* CIRM-BIA129 to Cow's Milk *Versus* Soymilk Environments Modulates Its Stress Tolerance and Proteome

OPEN ACCESS

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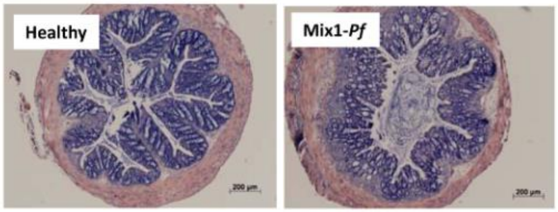
Growing demand for plant-based fermented products…

Consumer habits evolution
Vegan & flexitarian ↗

Health problems
Inflammatory Bowel Diseases (IBD)

Promising scientific results
Beneficial effects of fermented products on health

Foligné et al., 2016

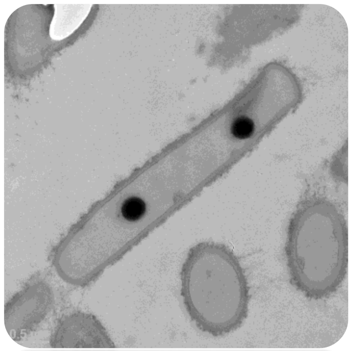


Development of probiotics fermented plant-based products

Fermentation →

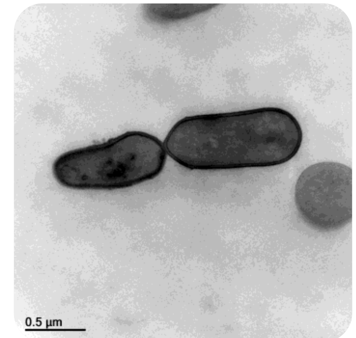
Well-known dairy probiotic bacteria used as starters

Lactobacillus delbrueckii* subsp. *bulgaricus



- One of the most used dairy starters
- Fermentation of yogurt and of diverse other fermented products, including cheeses

Propionibacterium freudenreichii



- Modulation of the gut microbiota, and inflammation
- Fermentation of diverse fermented products, including Emmental cheeses.

Little is known about their adaptation to the vegetable substrates

Aim of this study

To investigate the adaptation of these two probiotic bacteria to soymilk by comparison to cow milk.

Dairy probiotic starters adaptation to soymilk vs bovine milk

L. delbrueckii **subsp.** ***bulgaricus CIRM-BIA1592***

Bovine milk **Soy milk**


- Bacterial growth, alone and in co-culture with ***Streptococcus thermophilus*** **CIRM-BIA1345**
- Cell morphology
- Proteome composition

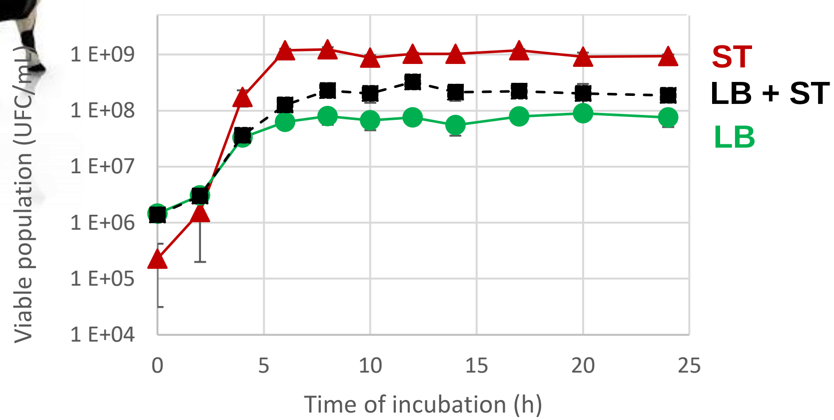
P. freudenreichii CIRM-BIA129

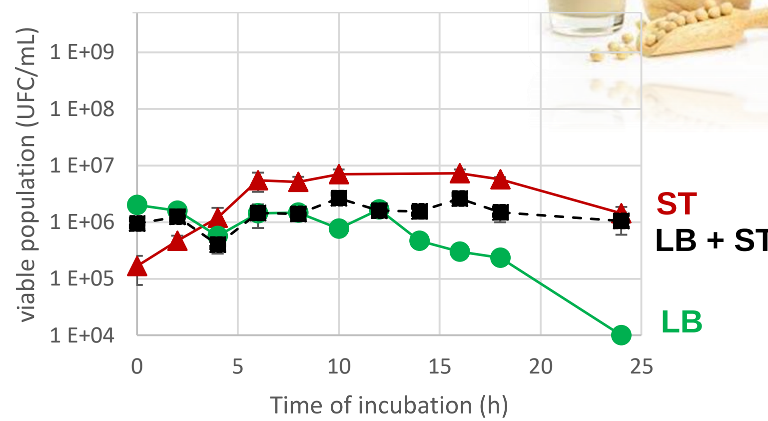
Bovine milk **Soy milk**


- Bacterial growth, alone and in co-culture with ***Lactobacillus plantarum*** **CIRM-BIA465**
- Cell morphology
- Proteome composition

L. delbrueckii bulgaricus in soymilk *vs* bovine milk

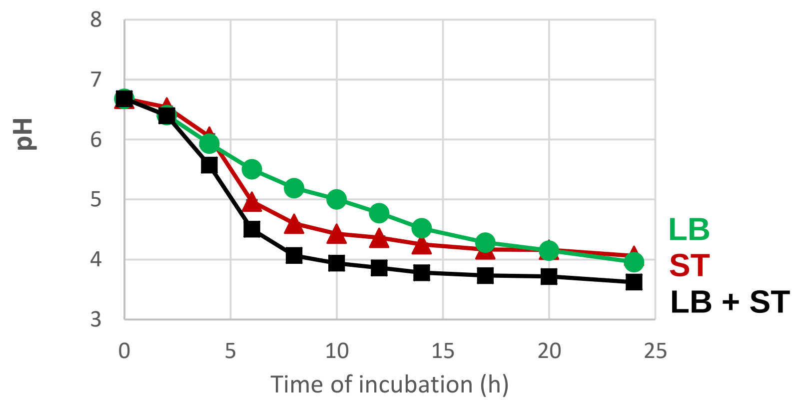
- Bacterial growth and substrate acidification

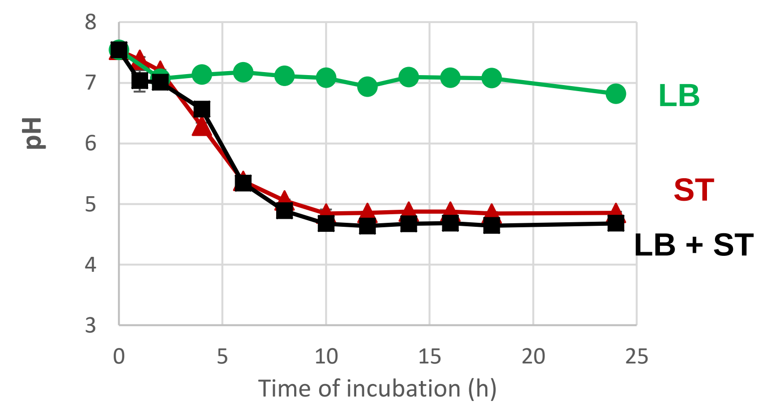




LB grew in cow milk.
Its growth was enhanced in co-culture with ST

LB did not grow in soy milk, neither alone, nor in co-culture with ST



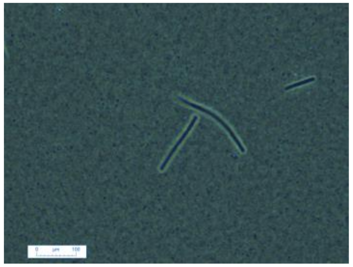


Acidification of bovine milk by LB
Faster acidification in co-culture with ST

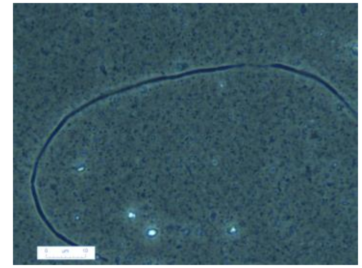
No acidification of soy milk by LB.
Faster acidification in co-culture with ST

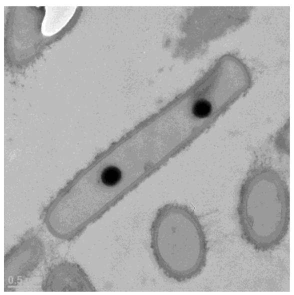
L. delbrueckii bulgaricus in soymilk *vs* bovine milk

- Cell morphology

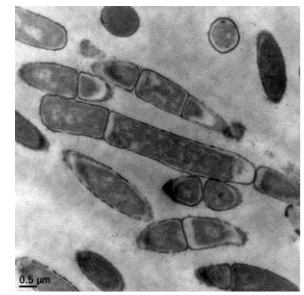


Optic microscopy



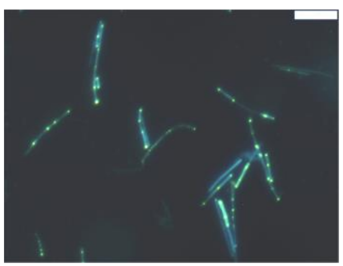


Electron microscopy

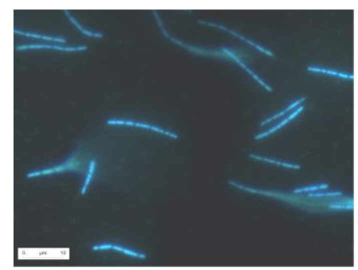


- **Long** and **straight** rods which appear **separate**

- **Short bacilli** comprised within **long and curved chains**



DAPI staining
&
Fluorescence microscopy



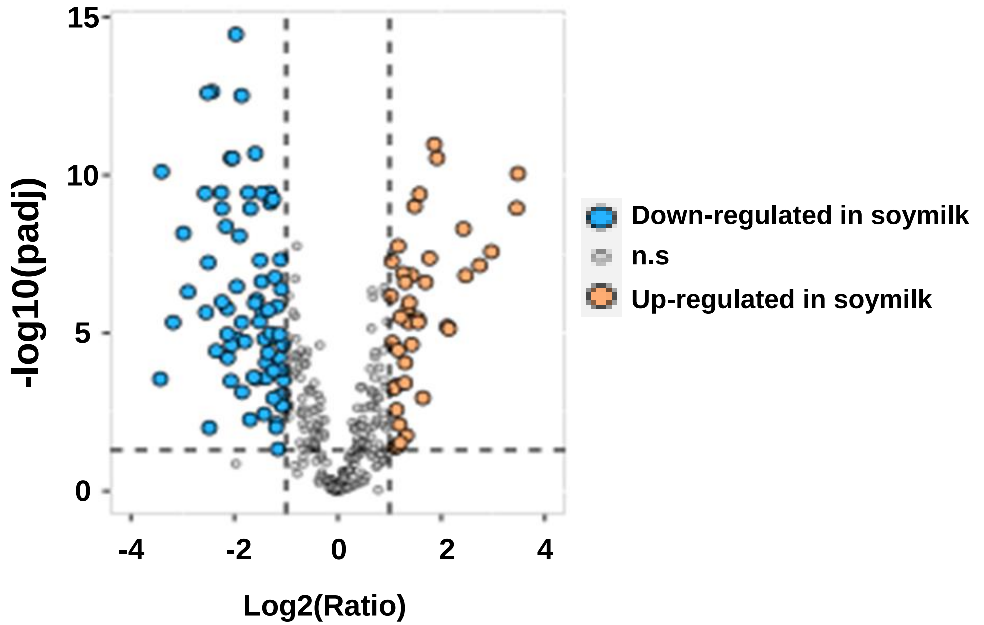
- **Straight rods**, with a **homogeneous blue fluorescence**
- Dots of **intense yellow fluorescence**
- **Presence of polyphosphate** under the form of granules at both ends of the lactobacilli cells

- **Long** and **curved rods** appeared segmented in **shorter segments**
- **No polyphosphate**
- DNA blue fluorescence was regularly distributed and compartmented within the long chains.

L. delbrueckii bulgaricus in soymilk *vs* bovine milk

- Proteome composition

Ldb **CIRM-BIA1592**



L. delbrueckii bulgaricus exhibits different proteomes in soy and in milk

- *185 proteins were differentially expressed:*

→ *75 were induced and 110 were repressed in soy*

L. delbrueckii bulgaricus in soymilk *vs* bovine milk

Proteome composition

Ldb **CIRM-BIA1592**

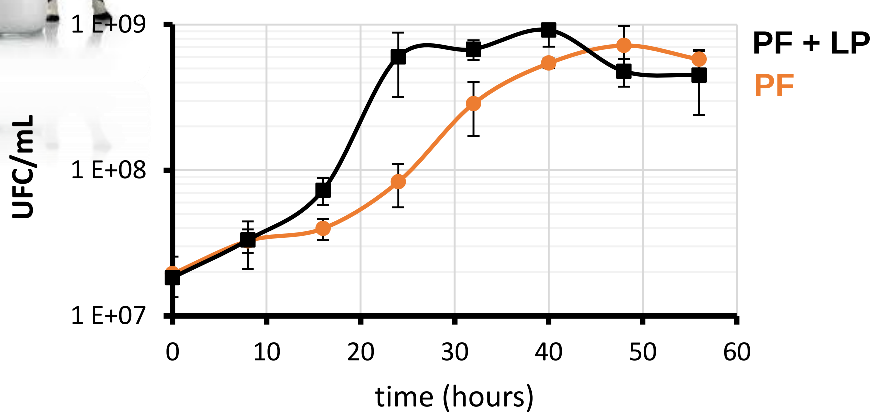
- **Carbohydrate transport and metabolism**
  - Fructose specific phosphotransferase system, fructose2,6-biphosphatase…
- **Energy production and conversion**
  - Fumarate metabolism
- **Amino acid transport and metabolism**
  - Branched-chain amino acid
- **Translation**
  - Ribosomal proteins, amino acid-tRNA ligases
- **Stress response proteins**
  - Catabolite control protein (CcpA), GreA transcription factor,…

- **Cell cycle and division**

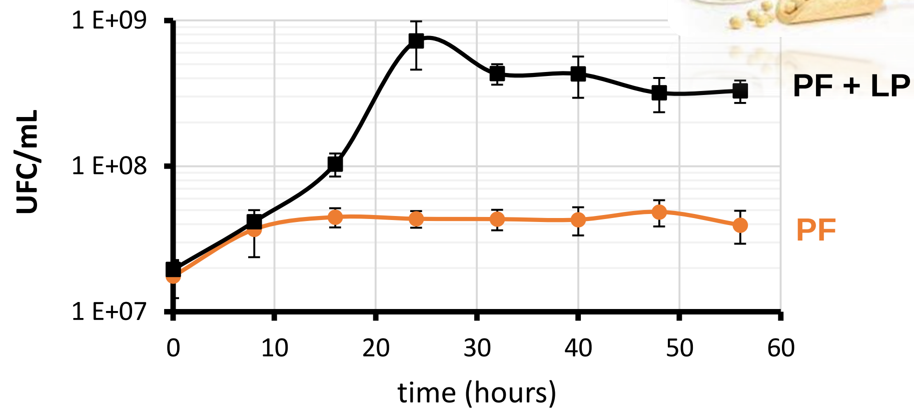
- Soy environment was non optimal for the growth of the yogurt starter *L. delbrueckii* subsp. *bulgaricus*.
- The development of new fermented products, based on soy milk, may require different microbial starters (others strains and/or species) more adapted to this substrate

P. freudenreichii in soymilk *vs* bovine milk

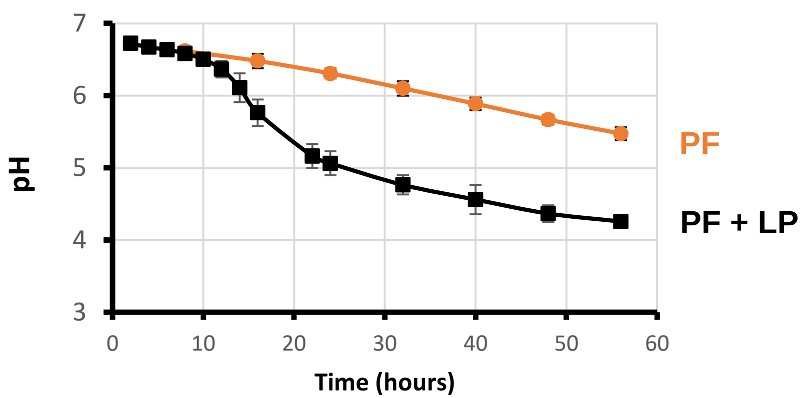
- Bacterial growth and substrate acidification



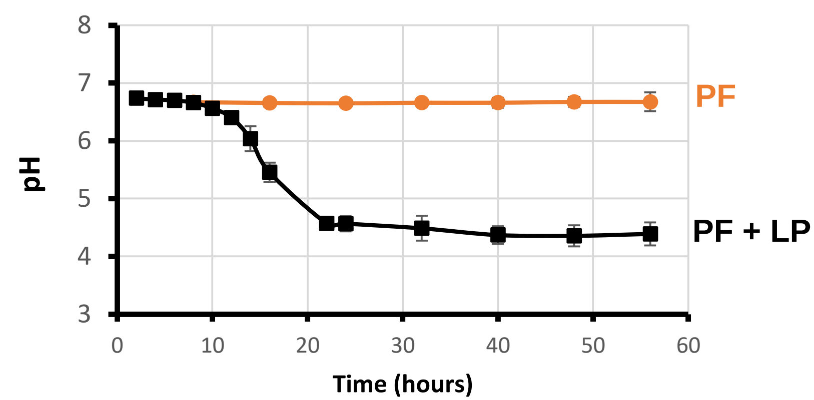
PF grew in cow milk.
Its growth was enhanced in co-culture with LP



PF alone did did not grow in soy milk.
Its growth was facilitated in co-culture with LP



Acidification of bovine milk by PF
More pronounced acidification in co-culture with LP

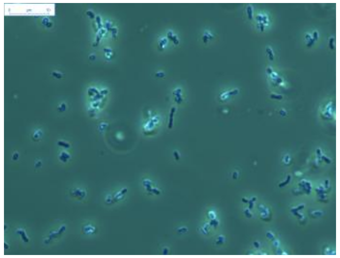


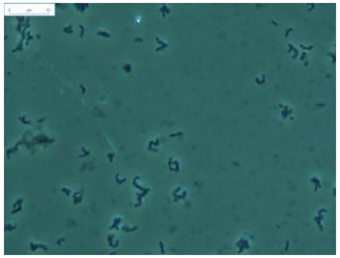
PF alone did not acidify soy milk.
Acidification was facilitated in co-culture with LP

→ Collaboration between PF and LP in terms of growth and metabolism in soymilk.

P. freudenreichii in soymilk *vs* bovine milk

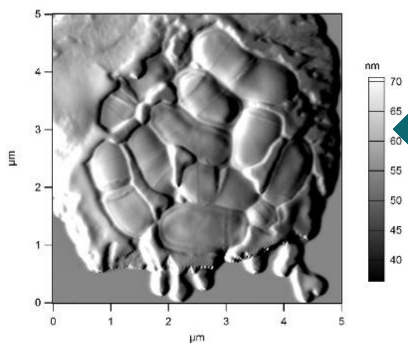
- Cell morphology

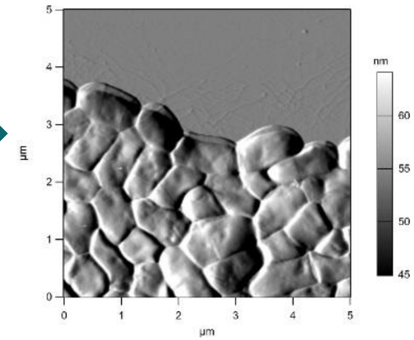




Optic microscopy

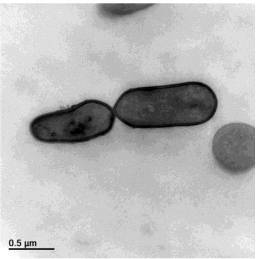
- Refringent and shiny aspect around propionibacteria in milk but not in soy

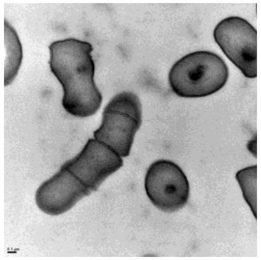




Atomic force microscopy three dimensional amplitude

- Surrounded by an extracellular compound in milk but not in soy



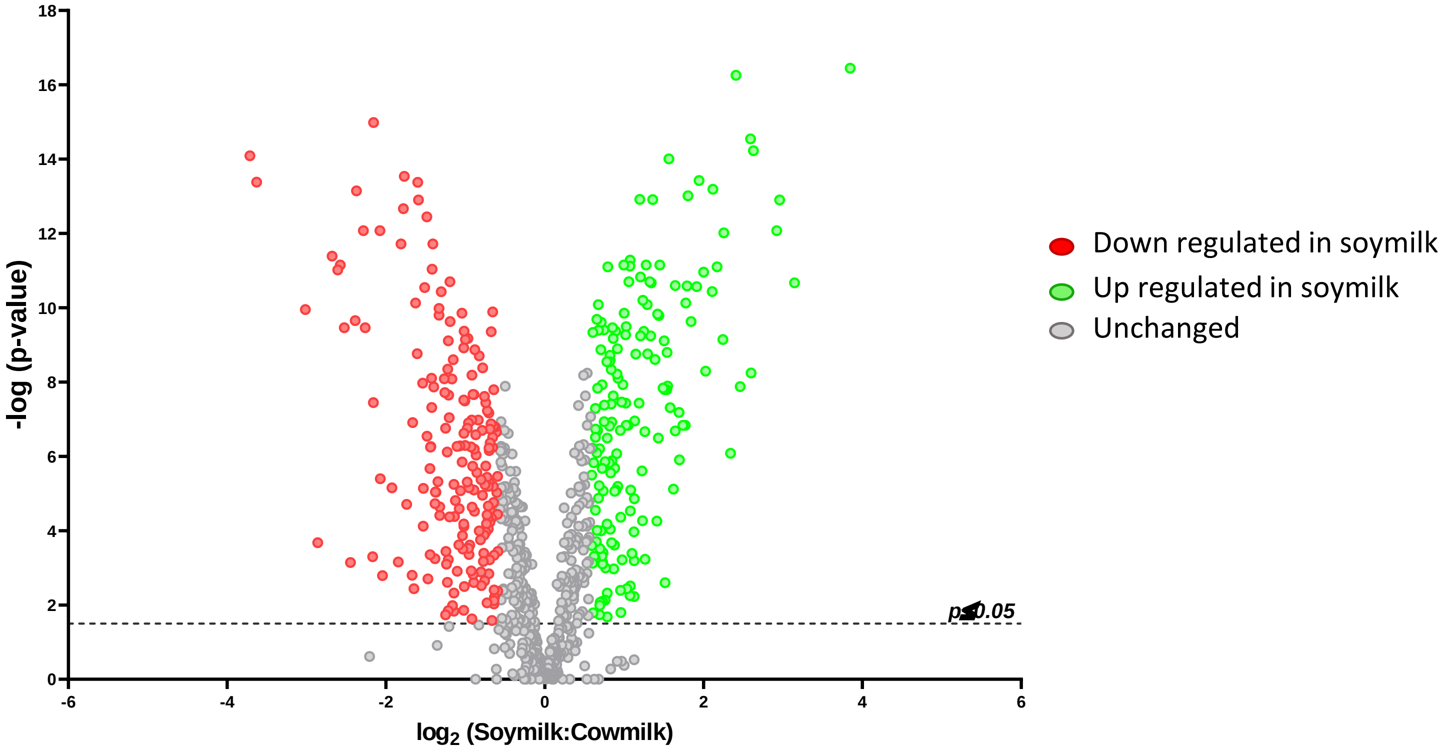


Electron microscopy

- Round-shaped *vs* in rectangular-shaped with less clearly defined cell wall limits

P. freudenreichii in soymilk *vs* bovine milk

- Proteome composition

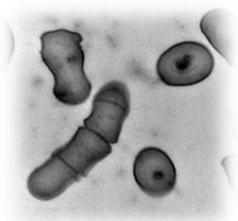


PF exhibits different proteomes in soy and in milk

- 374 *proteins were differentially expressed:*
  → *175 were induced and 199 were repressed in soy milk*

P. freudenreichii in soymilk *vs* bovine milk

▪ Proteome composition



***Pf* CIRM-BIA129**

- ❑ **Carbohydrate transport and metabolism; N=26**
  - Glycolysis, pentose phosphate pathway, myo-inositol utilization, …
- ❑ **Energy production and conversion, N=38**
  - Glycolysis, TCA cycle
- ❑ **Amino acid transport and metabolism; N=10**
- ❑ **Proteins involved in envelope biogenesis & cell wall construction**

- ❑ **Amino acid transport and metabolism; N=26**
- ❑ **Translation, ribosomal structure and biogenesis; N=34**
- ❑ **Heat and acid stress proteins**
- ❑ **S-layer proteins**

Probiotic abilities may be affected
(stress tolerance, persistence, immunomodulation)

In-vivo tests ?

- ❖ Changing the fermented substrate may thus significantly affect the **fermentative and probiotic properties** of dairy starters.
- ❖ This needs to be considered when developing new fermented functional foods.

Ongoing research work *(Illikoud et al. in preparation)*

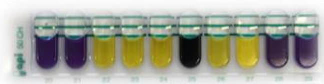
Technological criteria

Screening

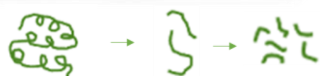
104 tested strains
8 reference strains
LAB & PAB

Anti-inflammatory properties

Veg. milk acidification



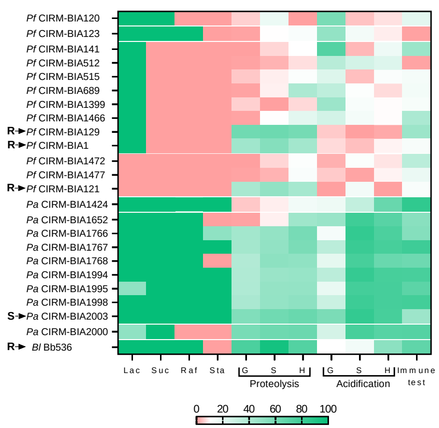
Carbohydrate utilization



Veg. protein hydrolyzis

Immunomodulation

Peripheral Blood Mononuclear cells (PBMCs)



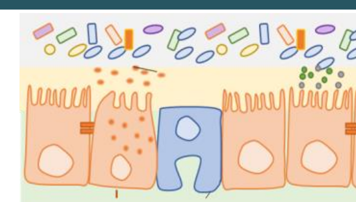
PAB cluster

LAB cluster

5 pre-selected probiotic starters

Pure strains & Bacterial consortia

Challenge in HEICs
Pro/anti-inflammatory cytokines



Human Epithelial Intestinal Cells

Thanks for your attention !

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