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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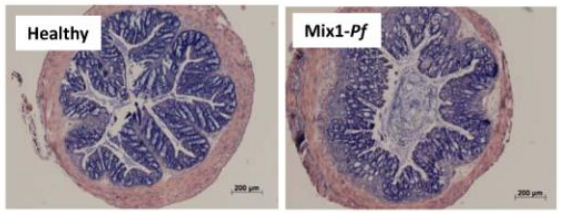
Growing demand for plant-based fermented products…

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

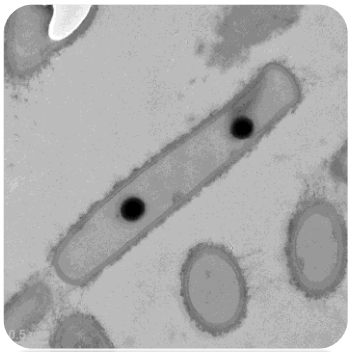


Development of probiotics fermented plant-based products

Fermentation

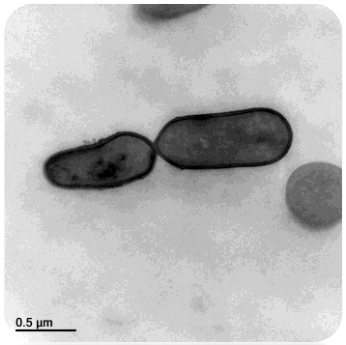
Well-known dairy probiotic bacteria used as starters

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

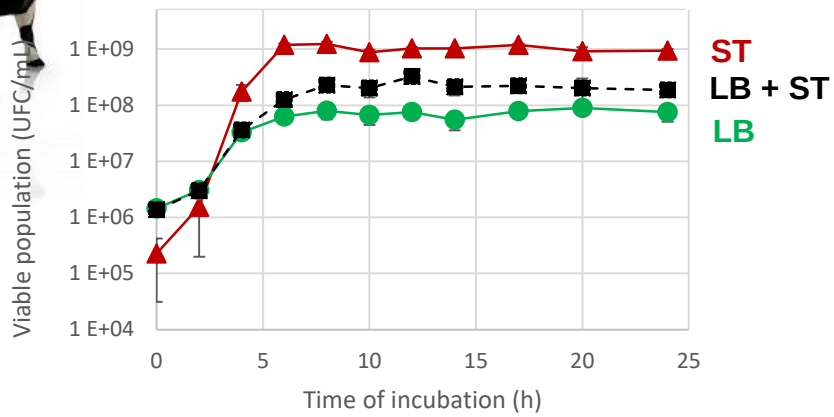
P. freudenreichii CIRM-BIA129

Bovine milk **Soy milk**

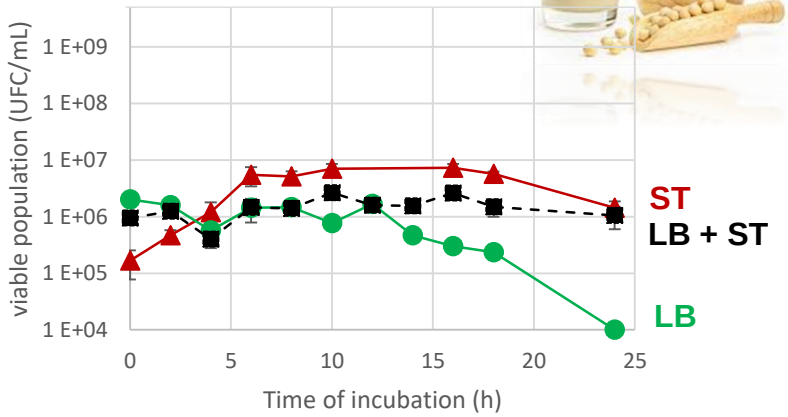
- Bacterial growth, alone and in co-culture with ***Lactiplantibacillus plantarum*** **CIRM-BIA465**
- Cell morphology
- Proteome composition

L. delbrueckii bulgaricus in soymilk *vs* bovine milk

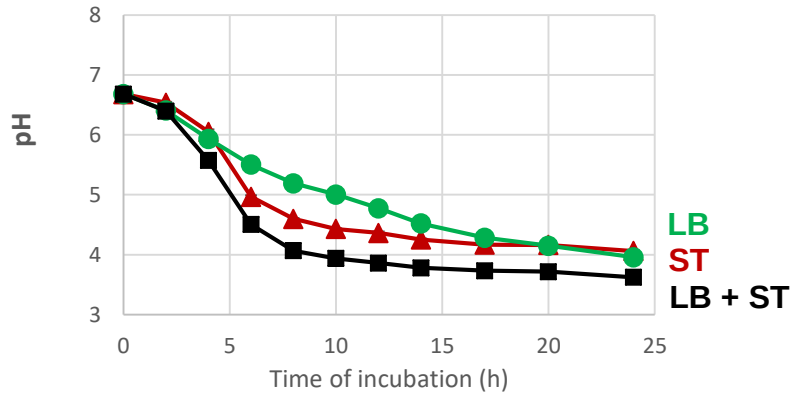
- Bacterial growth and substrate acidification



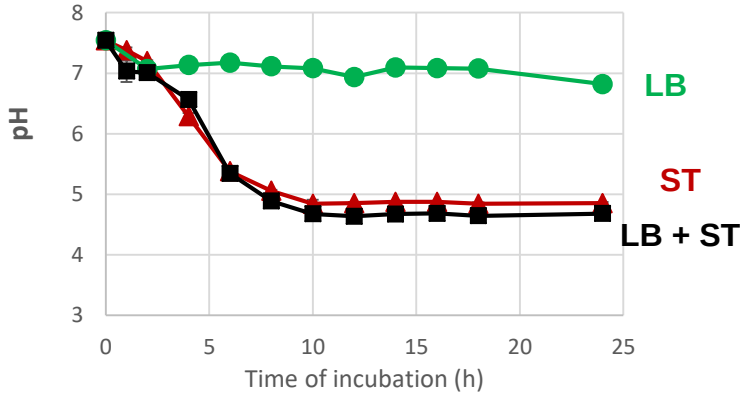
LB grew in cow milk.
Its growth was enhanced in co-culture with ST



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



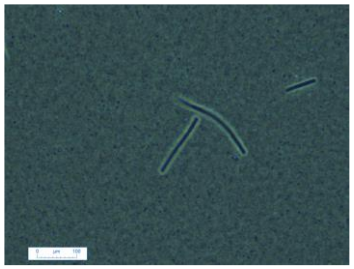
Acidification of bovine milk by LB
Faster acidification in co-culture with ST



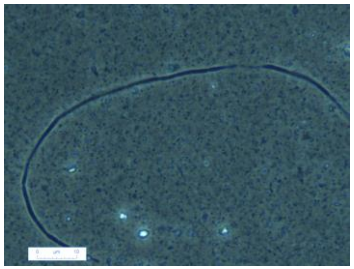
No acidification of soy milk by LB.
Acidification of soy milk in co-culture with ST

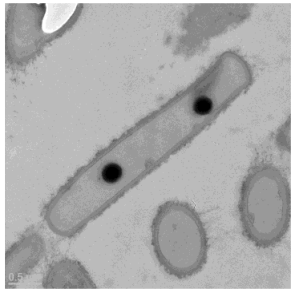
L. delbrueckii bulgaricus in soymilk *vs* bovine milk

- Cell morphology



Optical microscopy

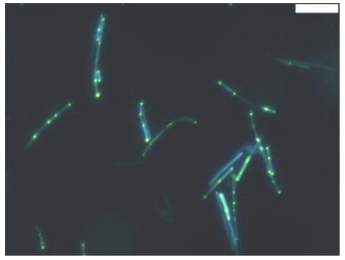




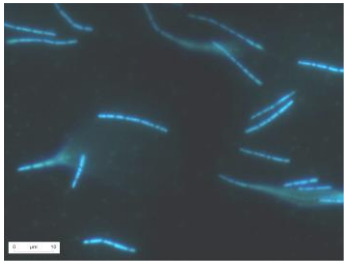
Electron microscopy

- **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

75 induced proteins

***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

Essential for cell viability

- **Stress response proteins**
  - Catabolite control protein (CcpA), GreA transcription factor,…
  - Proteins involved in protein turnover (Chaperone DnaJ)

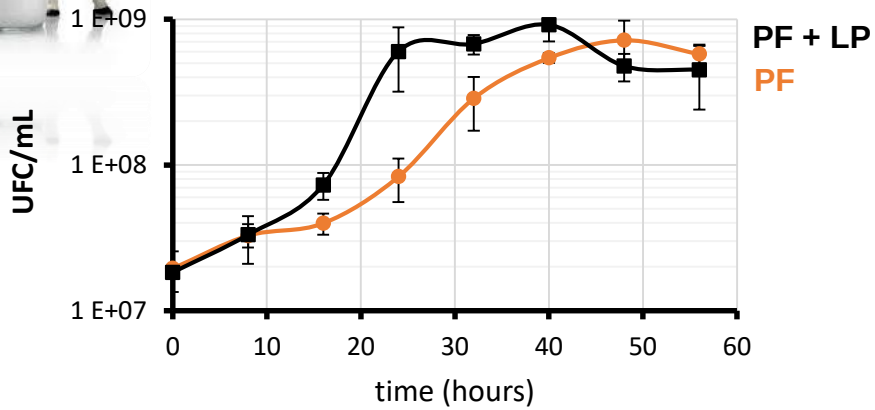
110 repressed proteins

- **Cell cycle and division**
  - **Cell Division protein (FitZ)**
  - **Peptidoglycan biosynthesis and cell wall biogenesis**

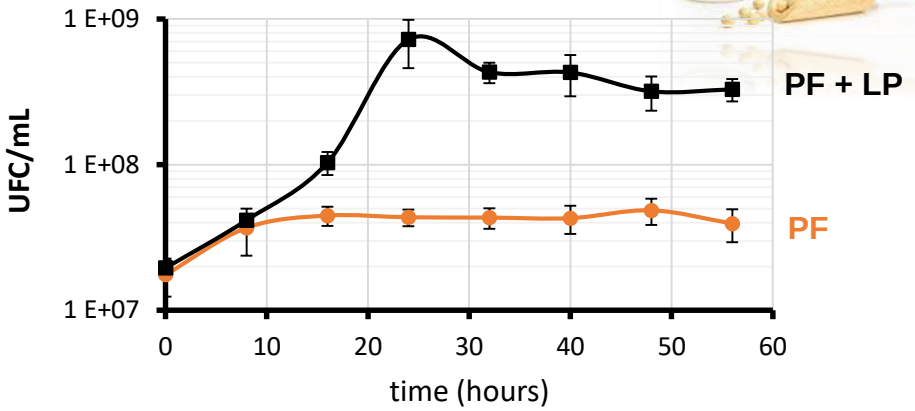
- Soy environment was non optimal for the growth of the yogurt starter *L. delbrueckii* subsp. *bulgaricus*.
- The development of new fermented products, based on soy milk, may require different microbial starters (others strains and/or species) more adapted to this substrate

P. freudenreichii in soymilk *vs* bovine milk

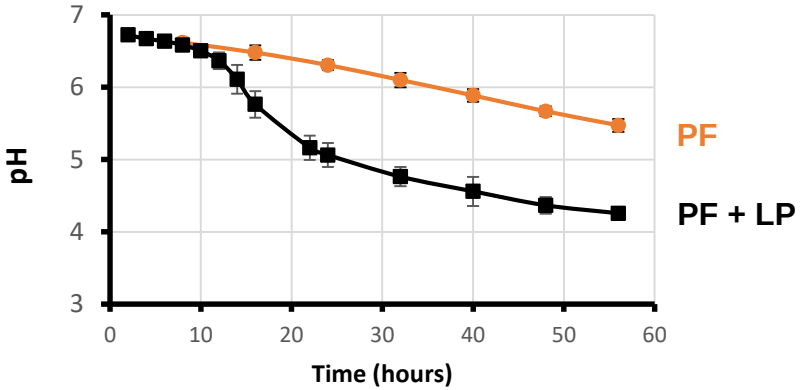
- Bacterial growth and substrate acidification



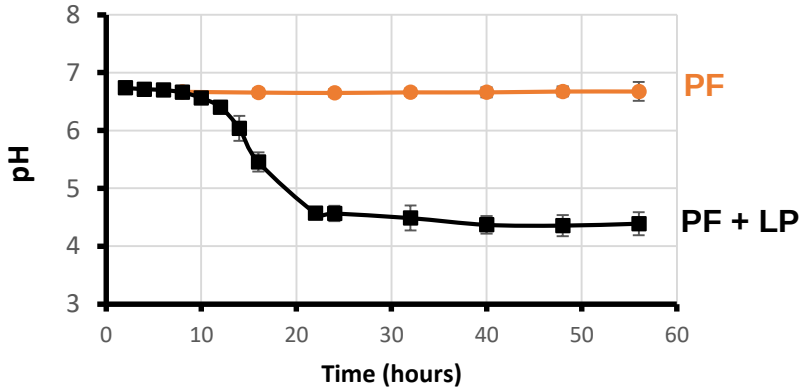
PF grew in cow milk.
Its growth was enhanced in co-culture with LP



PF alone did 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

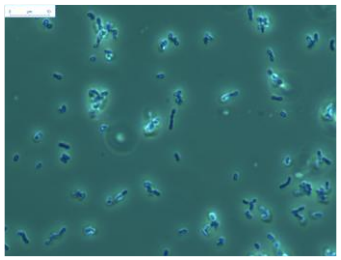


PF alone did not acidify soy milk.
Acidification was facilitated in co-culture with LP

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

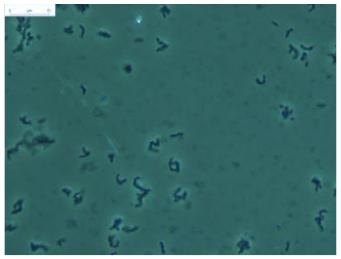
P. freudenreichii in soymilk *vs* bovine milk

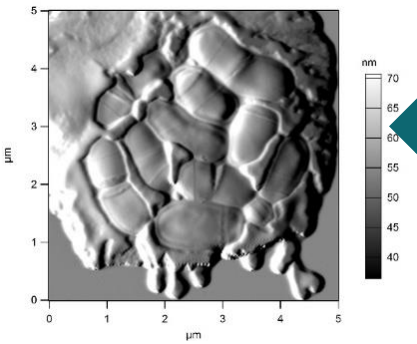
- Cell morphology



Optical microscopy

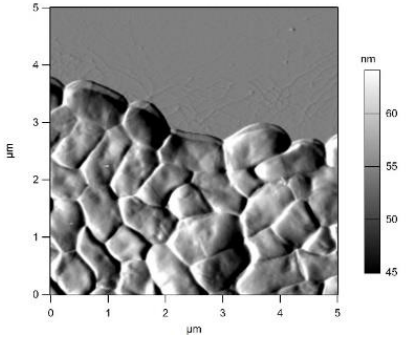
- Refringent and shiny aspect around propionibacteria in milk but not in soy

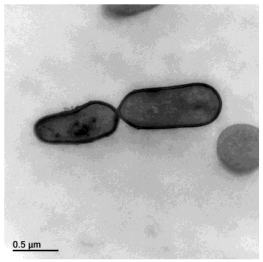




Atomic force microscopy three dimensional amplitude

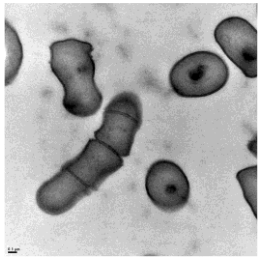
- Surrounded by an extracellular compound in milk but not in soy





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* CIRM-BIA129**

175 induced proteins

- **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**
  - Ser, Ala, Gly and derivates present in soy
- **Proteins involved in envelope biogenesis & cell wall construction**
- **stress adaptation proteins and enhanced stress tolerance**

199 repressed proteins

- **Amino acid transport and metabolism; N=26**
- **Translation, ribosomal structure and biogenesis; N=34**
- **Post-translational modification, protein turnover and chaperones**
  - Heat and acid stress proteins
  - S-layer proteins

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

- 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*)

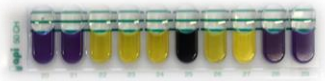
Screening

104 tested strains
8 reference strains
LAB & PAB

Technological criteria

Anti-inflammatory properties

Veg. milk acidification

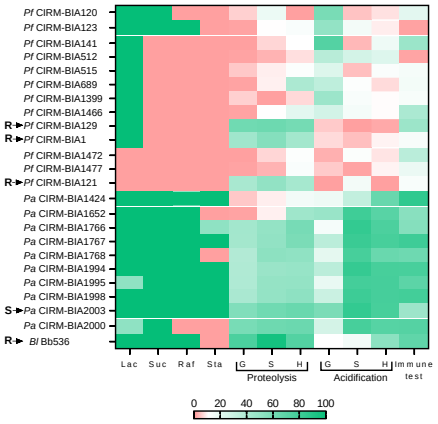


Carbohydrate utilization

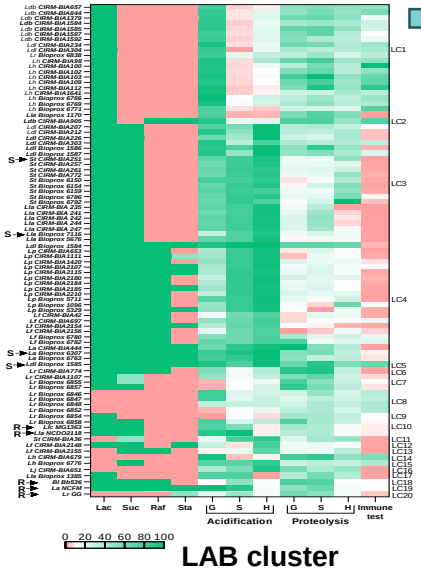
Veg. protein hydrolyzis

Immunomodulation

Peripheral Blood Mononuclear cells (PBMCs)



PAB cluster



LAB cluster

5 pre-selected probiotic starters

Pure strains & Bacterial consortia

Challenge in HEICs
Pro-inflammatory cytokines

Human Epithelial Intestinal Cells

Thanks for your attention !

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