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Do probiotic dairy starters adapt to vegetable milks?

Nassima Illikoud, Florian Tarnaud, Floriane Gaucher, Fillipe Luiz Rosa Do Carmo, Julien Jardin, Valérie Briard-Bion, Fanny Guyomarc'H, Valérie Gagnaire, Gwénaél Jan

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➤ Do probiotic dairy starters adapt to vegetable milks?

Nassima Illikoud

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

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➤ Growing demand for plant-based fermented products...

Consumer habits evolution
Vegan & flexitarian

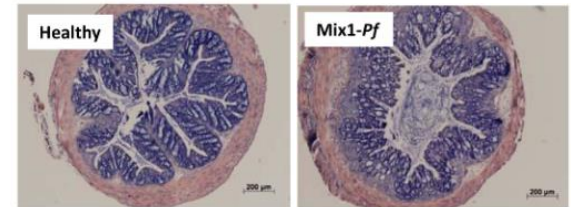


Health problems
Inflammatory Bowel
Diseases (IBD)



Promising scientific results
Beneficial effects of fermented
products on health

Foligné et al., 2016



Development of probiotics fermented plant-based products

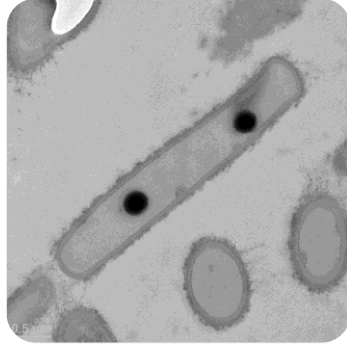


Fermentation

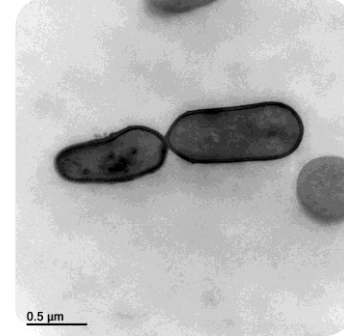


➤ Well-known dairy probiotic bacteria used as starters

Lactobacillus delbrueckii subsp. *bulgaricus*



Propionibacterium freudenreichii



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

- 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-BIA 1592

Bovine milk

Soy milk



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

P. freudenreichii CIRM-BIA 129

Bovine milk

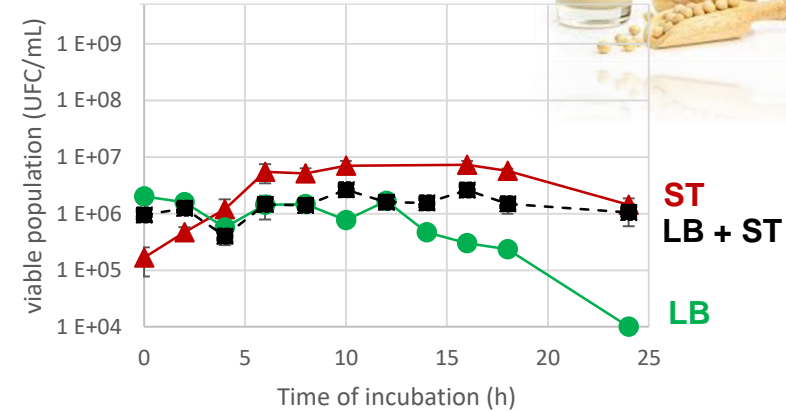
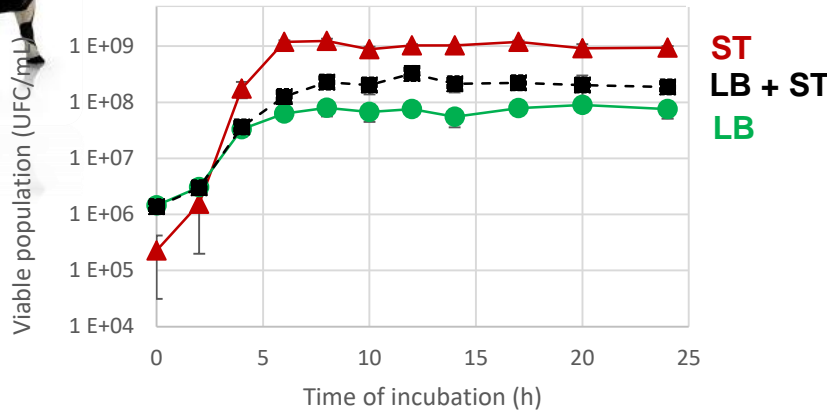
Soy milk



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

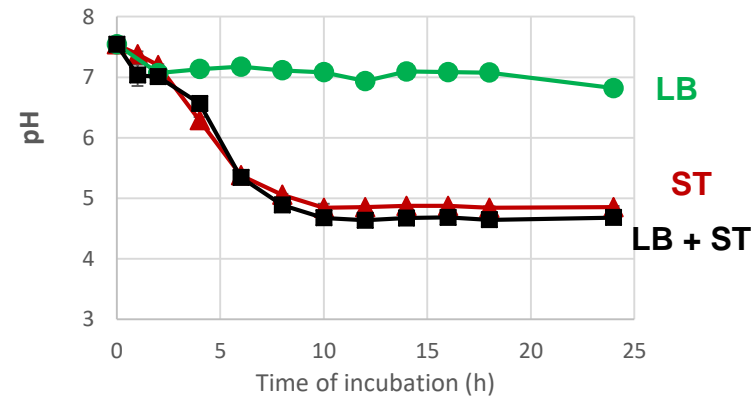
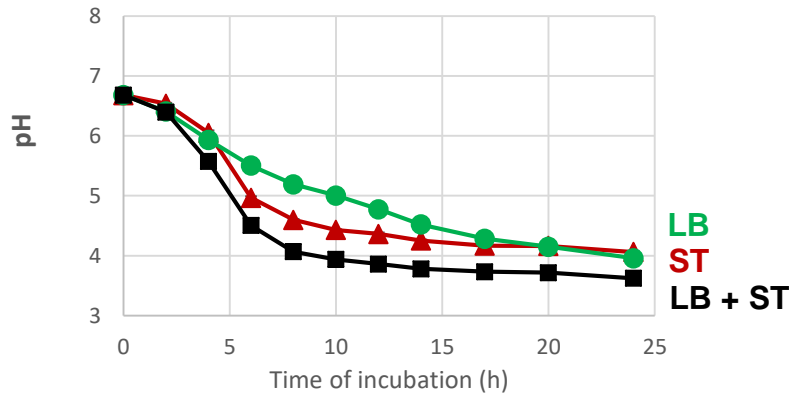
➤ *L. delbrueckii bulgaricus* in soymilk vs bovine milk

■ 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

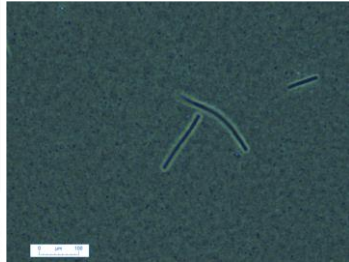


Acidification of bovine milk by LB
Faster acidification in co-culture with ST

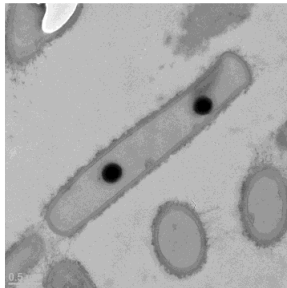
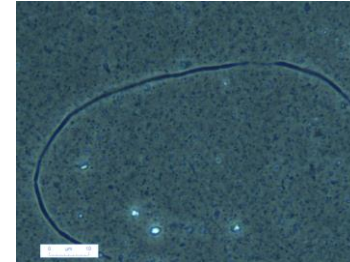
No acidification of soy milk by LB.
Faster acidification in co-culture with ST

➤ *L. delbrueckii bulgaricus* in soymilk vs bovine milk

■ Cell morphology



Optic microscopy

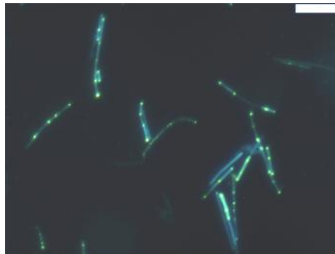


Electron microscopy

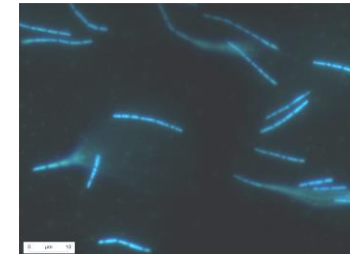


- Long and **straight** rods which appear **separate**

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



DAPI staining
&
Fluorescence microscopy

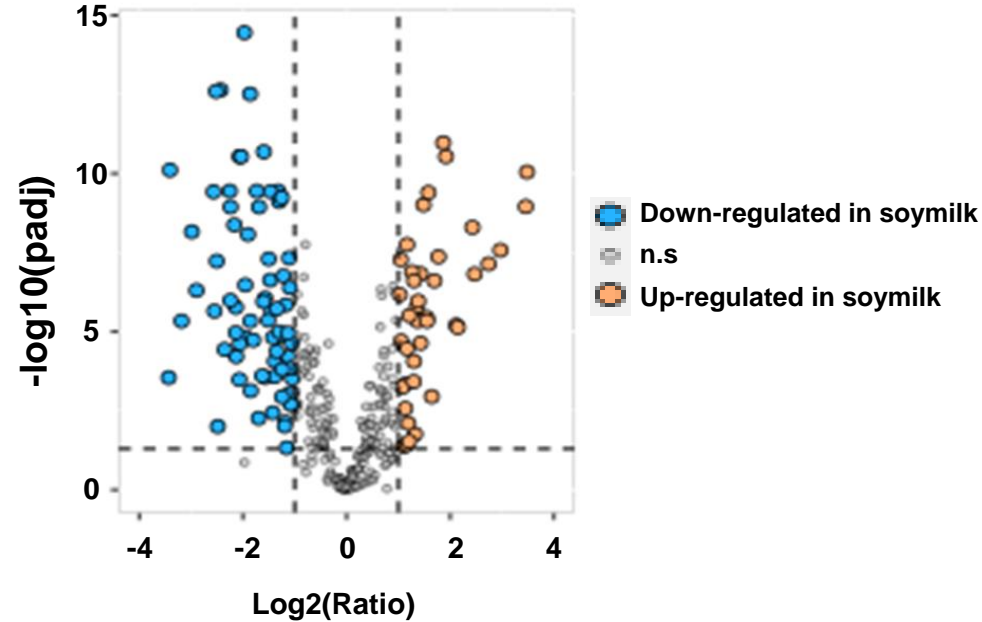


- **Straight rods**, with a **homogeneous blue fluorescence**
- Dots of **intense yellow fluorescence**
- **Presence of polyphosphate** under the form of granules at both ends of the lactobacilli cells

- **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



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**

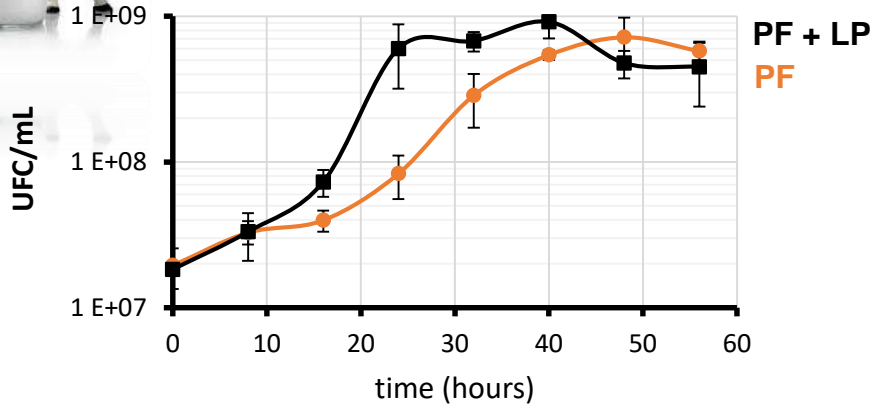
- ❖ Soy environment was non optimal for the growth of the yogurt starter *L. delbrueckii* subsp. *bulgaricus*.
- ❖ The development of new fermented products, based on soy milk, may require different microbial starters (others strains and/or species) more adapted to this substrate



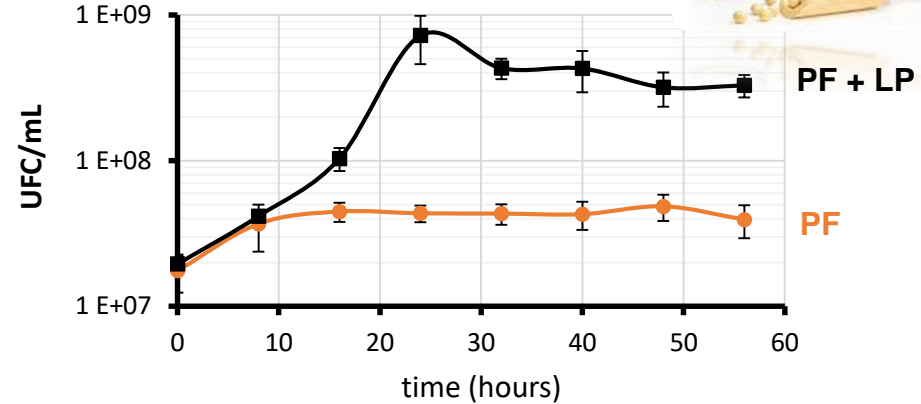
➤ *P. freudenreichii* in soymilk vs bovine milk



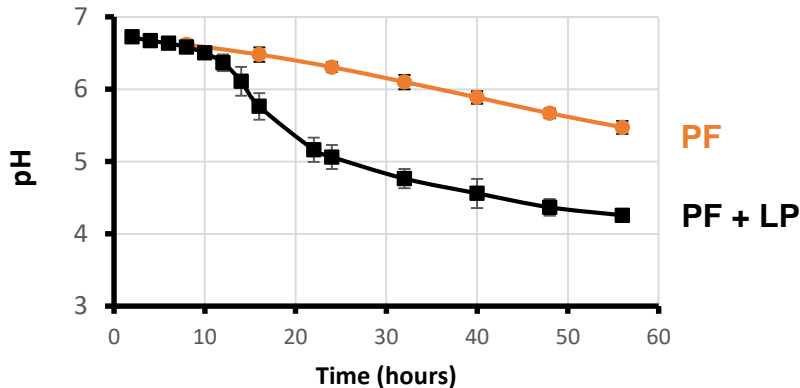
■ Bacterial growth and substrate acidification



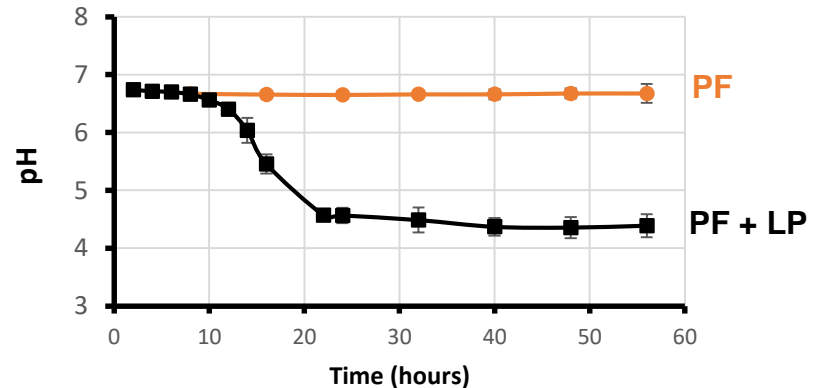
PF grew in cow milk.
Its growth was enhanced in co-culture with LP



PF alone did 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

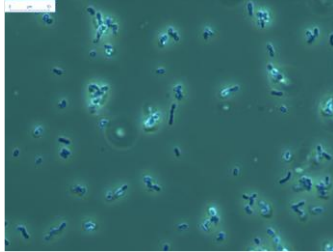


PF alone did not acidify soy milk.
Acidification was facilitated in co-culture with LP

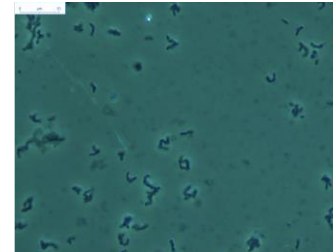
➔ Collaboration between PF and LP in terms of growth and metabolism in soymilk.

➤ *P. freudenreichii* in soymilk vs bovine milk

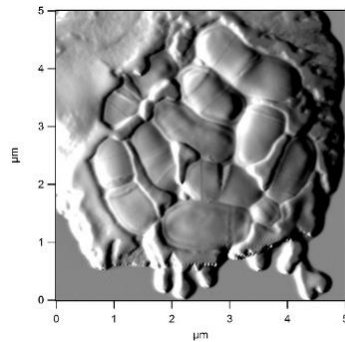
▪ Cell morphology



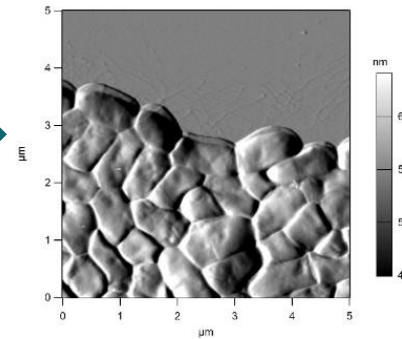
Optic microscopy



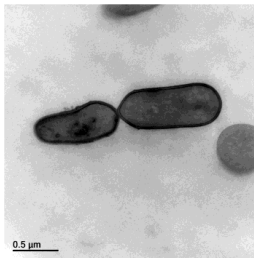
- Refringent and shiny aspect around propionibacteria in milk but not in soy



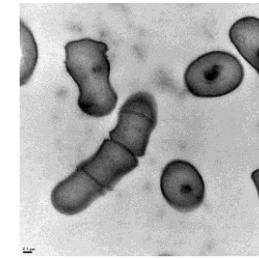
Atomic force microscopy three dimensional amplitude



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



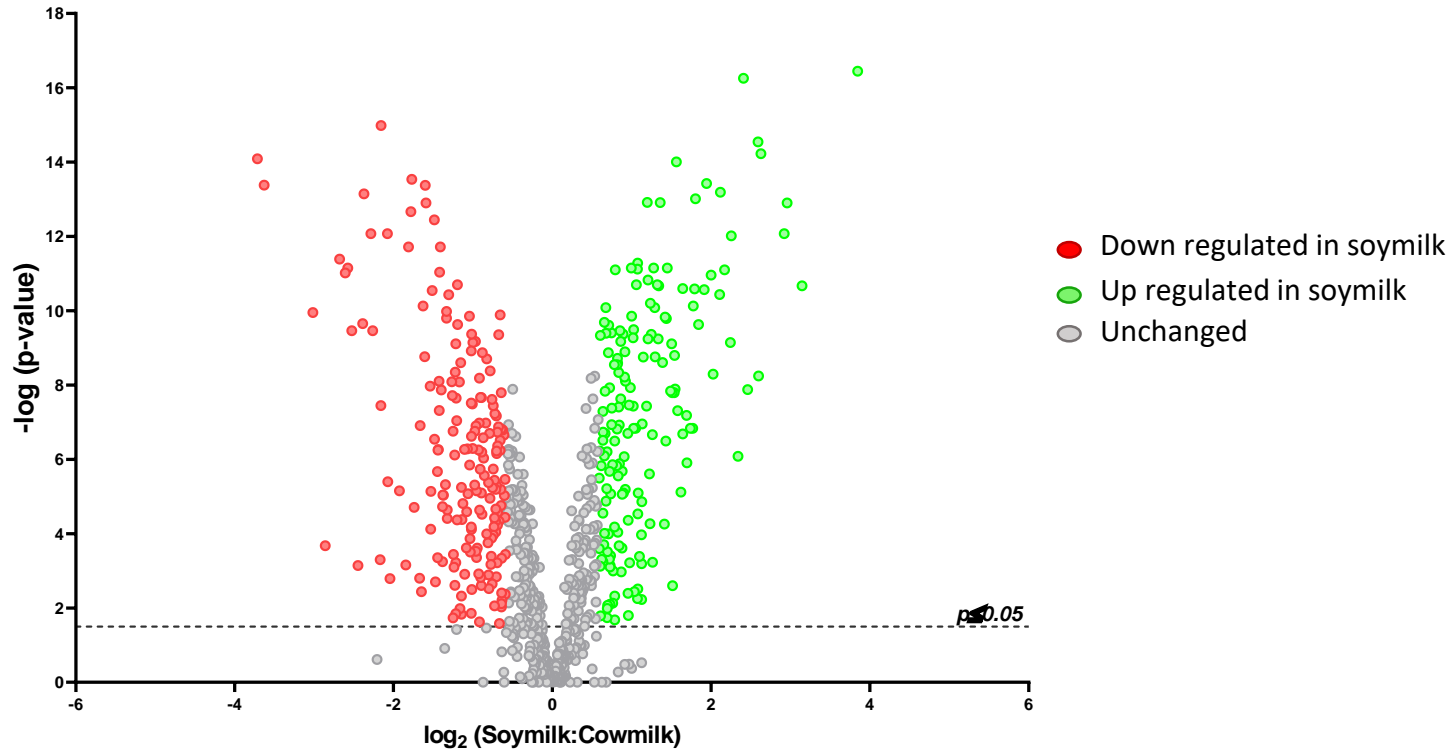
Electron microscopy



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

➤ *P. freudenreichii* in soymilk vs bovine milk

■ Proteome composition

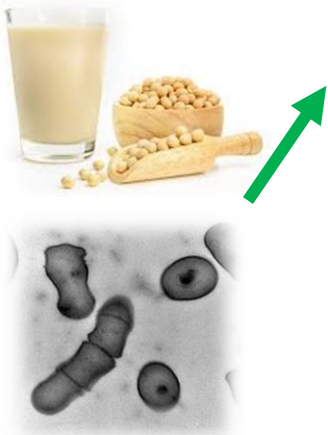


PF exhibits different proteomes in soy and in milk

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

➤ *P. freudenreichii* in soymilk vs bovine milk

■ Proteome composition



- ❑ **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**

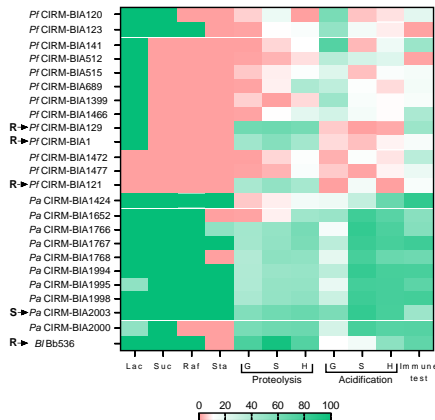
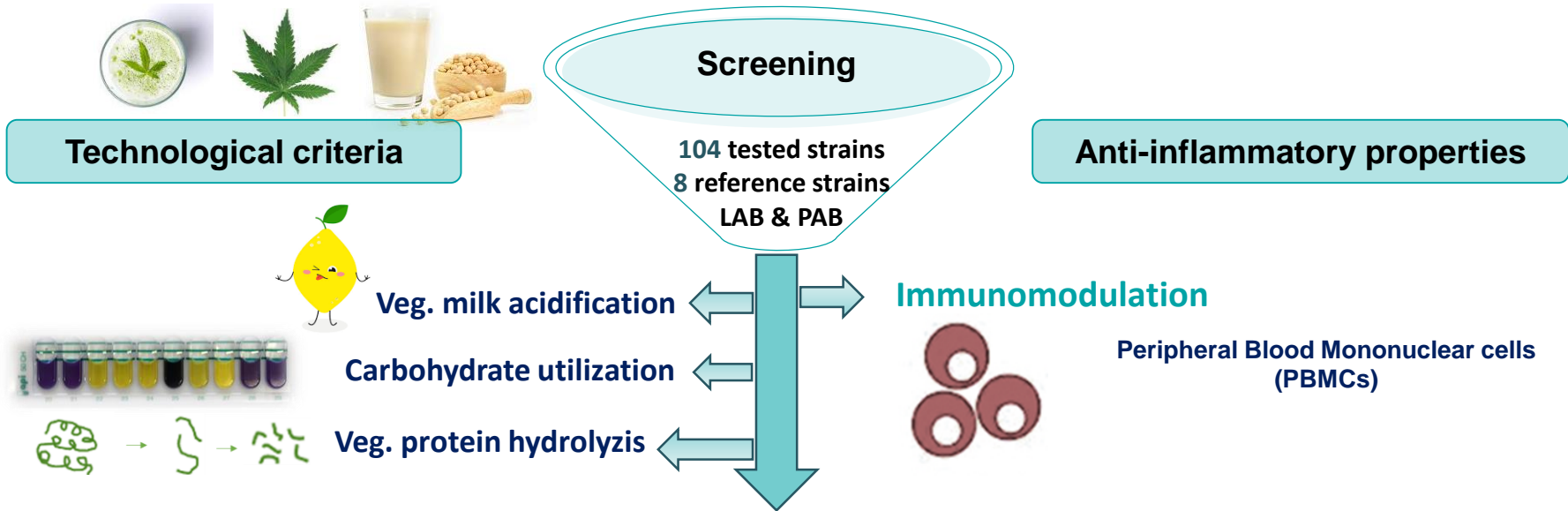
- ❑ **Amino acid transport and metabolism; N=26**
- ❑ **Translation, ribosomal structure and biogenesis; N=34**
- ❑ **Heat and acid stress proteins**
- ❑ **S-layer proteins**

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*)



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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