

Innovative use of sweet whey to improve the viability of spray-dried probiotic bacteria



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Science & Technology of Milk & Egg

83 standing fellow workers

25 PhD students



**A multidisciplinary and multiscale approach,
reinforced by two high-calibre facilities:**

Dairy Platform



Biological Resource Centre



- ❑ **Structuration / destructuration mechanisms of food matrix:** *from structural characterisation to digestion*
- ❑ **Dairy processing and cheese making,** *toward sustainable dairy process engineering*
- ❑ **Microbial interaction:** *food matrix and host cell*



1.

- **Background**

2.

- **A Novel Process**

3.

- **Underlying Mechanisms**

4.

- **Scale-up and Application**

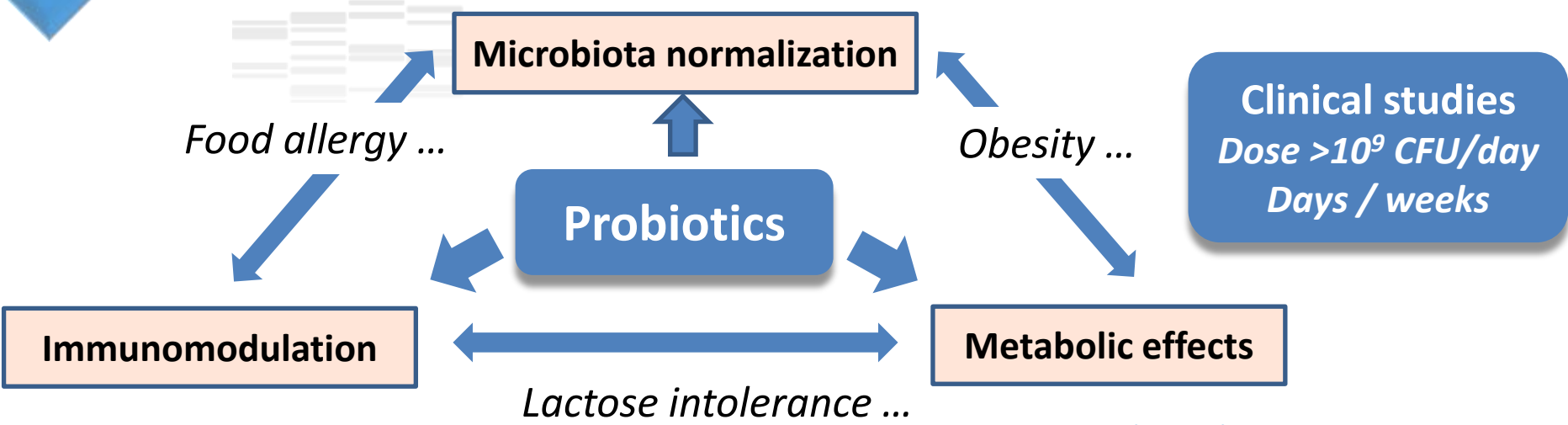
5.

- **Conclusion and Perspectives**

1.

• Background

Probiotic benefits and market



Nat Rev Endocrinol. 2011, 7: 639-646
Lancet. 2007, 369: 1614-1620

Probiotics global market

~ 64,6 billion\$ in 2020



> 7,4%
compound
annual growth
rate

36.6 billion\$ in 2015

Market share (companies)

Danone	}	< 40% (2015)
Yakult		
Nestle		
Danisco		
Chr. Hansen		

Markets and Markets, 2015; Global Market Insights, 2016; Mordor Intelligence, 2016



IMF market

- **Sustained by globalization**, economical transition and population growth
- From 2007, **8-20% annual growth** → **2 million tons produced /year**
- **China ~ 50% market**
- Drives **80% of the European dairy investment** since 2011

Mimicking breast milk

g/l	Cow milk	Human milk
Proteins	32	10
<i>Caseins</i>	80%	35%
<i>WP</i>	20%	65%
Carbohydrates	50	75
Lipids	38	36
<i>PUFA</i>	2,9%	6,4%

Next generation

Incorporation of live probiotics

- key strategy to **reduce the incidence and severity of diarrhea** in infants
- Improves **intestinal health markers**
→ Needs for a **better characterization of breast microbiota for probiotics selection**

Curr. Opin. Biotechnol. 2014, 26, 141–147

Nutrients 2016, 8, 279-289

Pediatric Research 2017, 81, 622-631

1.

• Background

Probiotic production



Typical freeze drying cycle



Arnab Ganguly, Alexeenko, et al., 2010

Dry / powder form

- **Stable / encapsulated** in the dry matrix
- Support **long storage** and more convenient for transport
- **Easier dosage** in foods

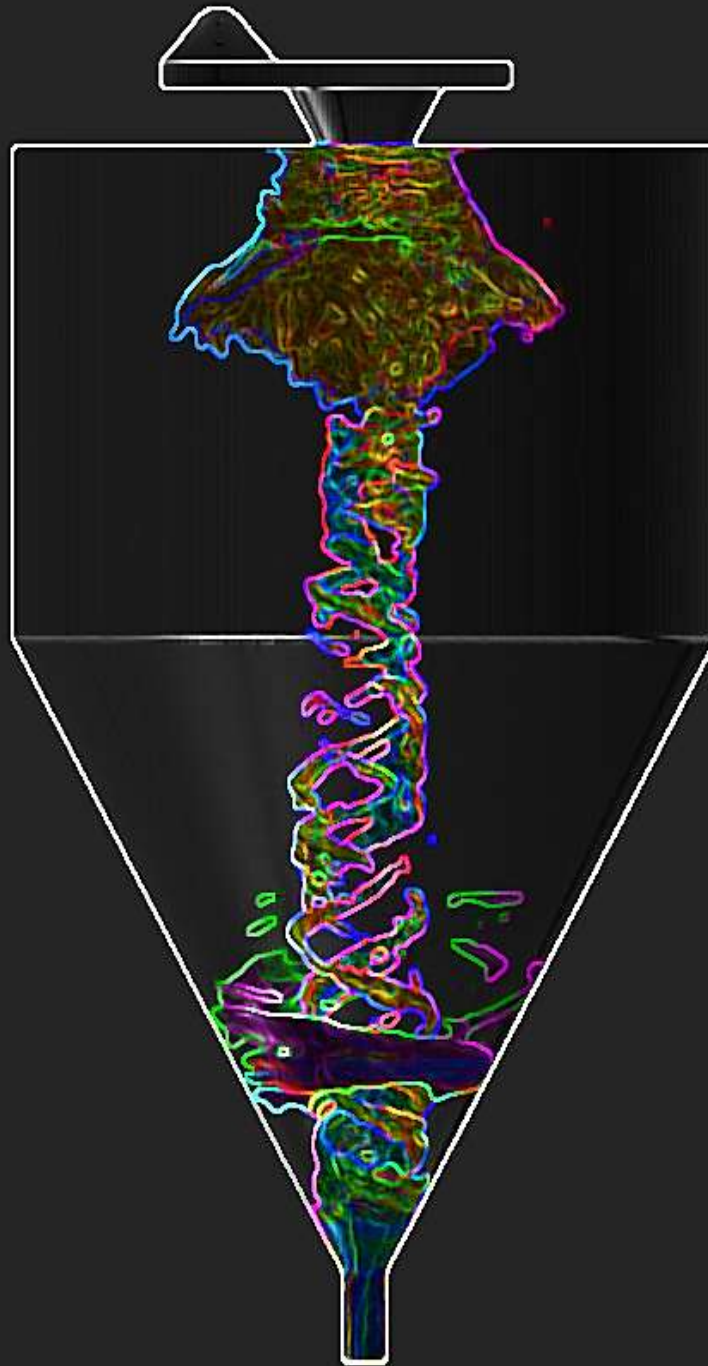
Mostly through freeze drying

- **Efficient / high probiotics viability**
- **Discontinuous**
- **Low productive**
- **High energy cost**: 10 MWh / ton water evaporated

Unable to meet the growing market demand in a sustainable way

Spray drying

- ✓ Cost
- ✓ Continuous
- ✓ Productive
- ✓ Encapsulation



Challenges

- Temperature
- Oxygen
- Shear force
- Shrinkage
- Dehydration

Poor survival rates

1.

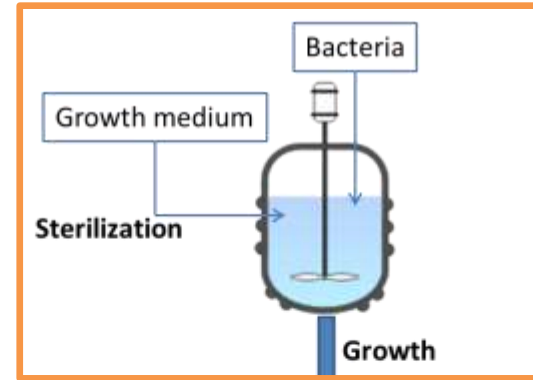
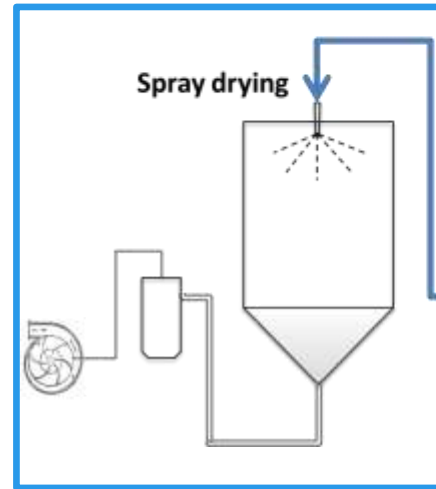
• Background

Current strategies in probiotics spray drying Research



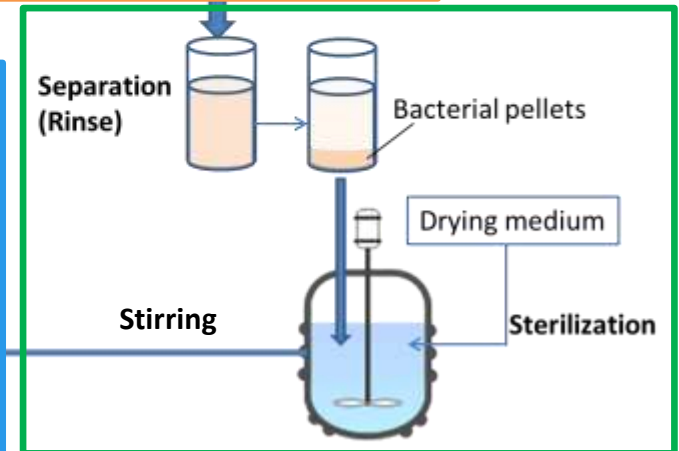
Multi-stage drying
Low-T techniques

...



Strain Selection
Cross-protection

...



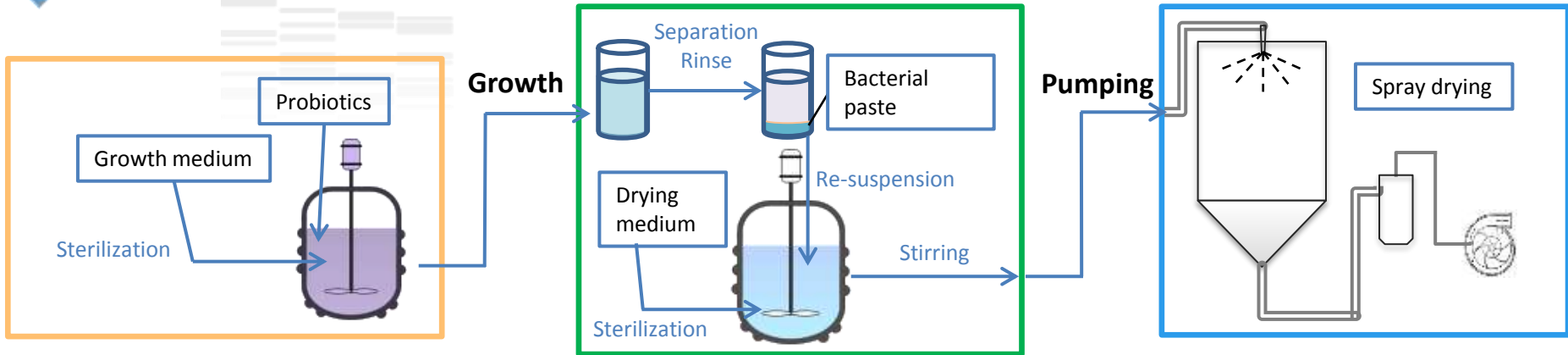
Carbohydrates
Proteins / Polymers
Minerals

...

2.

• A Novel Process

A new horizon



Cross-protection:

- Heat
- Osmotic
- Acid
- Oxidative
- ...

Food grade materials
 Carbohydrates
Concentrated 2-in-1 Medium
 Proteins
 Minerals
 ...

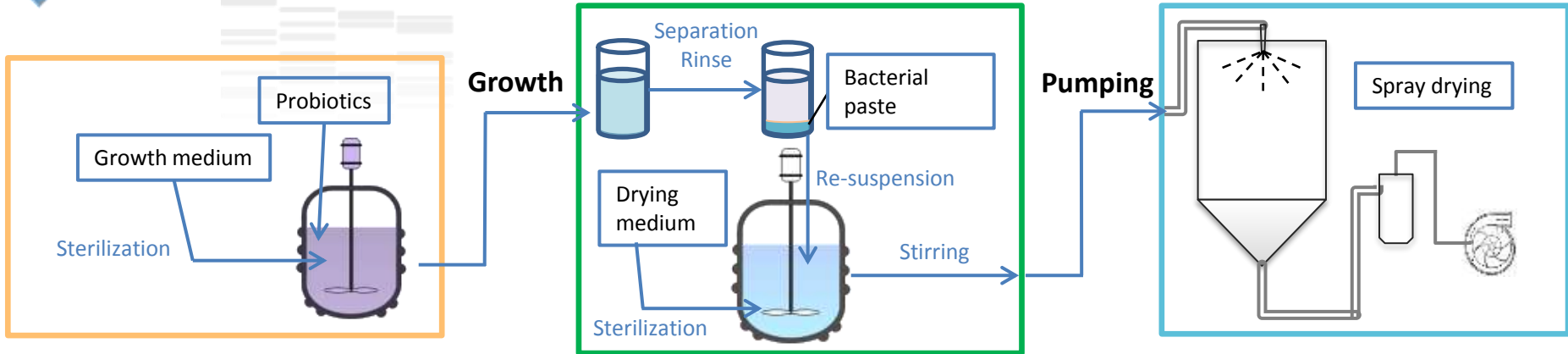
Multi-stage drying:

Pre-concentration

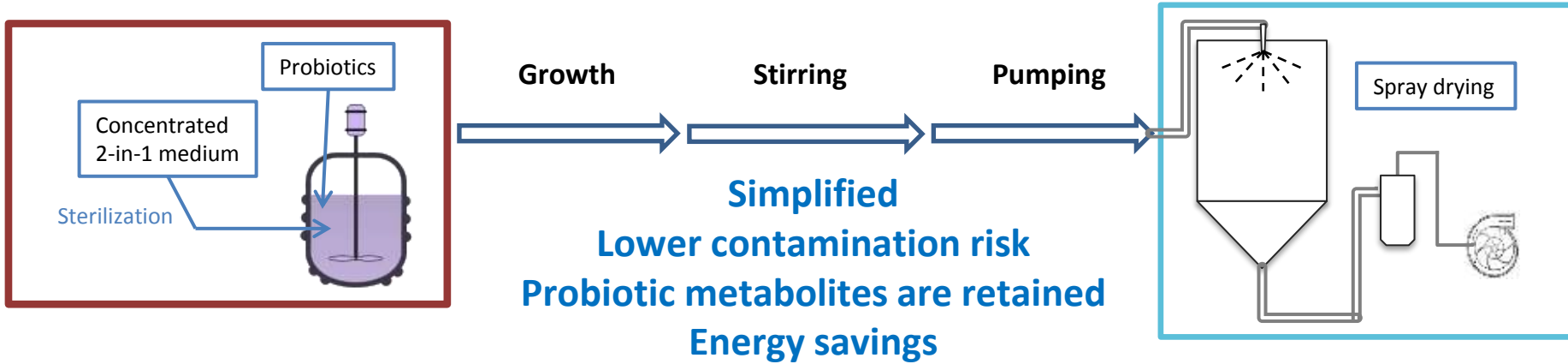
2.

A Novel Process

A new horizon



2-in-1 process



2.

A Novel Process

Sweet whey

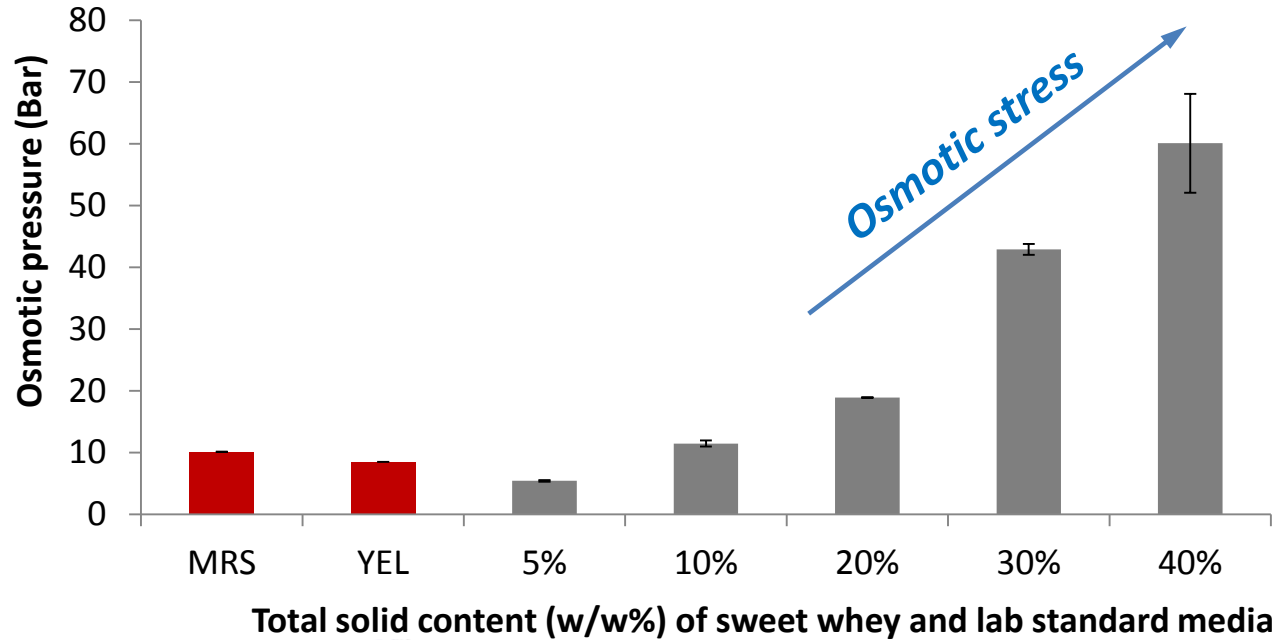
- Food grade
- Nutritive / protective
- Recombined at different concentration

Probiotic strains

- *L. casei* BL23 (fragile)
- *P. freudenreichii* 129 (robust)



2-in-1 medium and strains



Gut

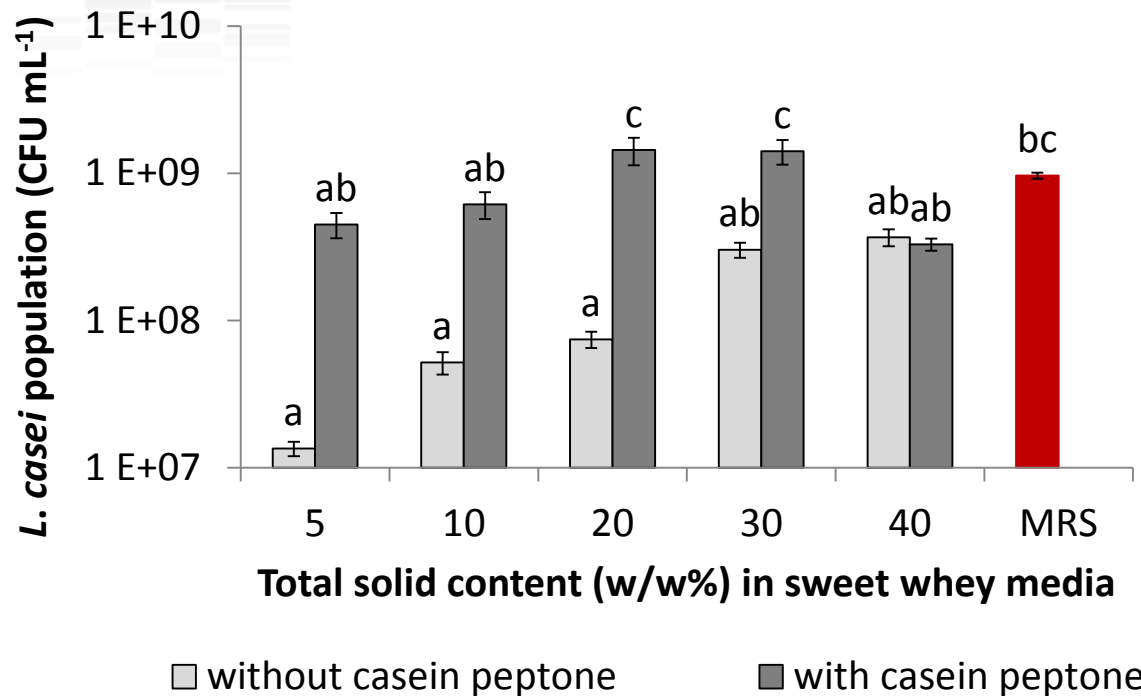
Molecular Nutrition

Microbial Cell Factories

Applied and Environmental Microbiology

Abstracts:

- Combining selected immunomodulatory *Propionibacterium freudenreichii* strains: 7 of an anti-inflammatory
- Anti-inflammatory effects of *Lactobacillus casei* BL23 producing or not a manganese-dependent catalase on DSS-induced colitis in mice
- Surface proteins of *Propionibacterium freudenreichii* are involved in its anti-inflammatory properties
- Milk Fermented by *Propionibacterium freudenreichii* Induces Apoptosis of HGT-1 Human Gastric Cancer Cells
- The probiotic *Propionibacterium freudenreichii* as a new adjunct for TRAIL-based therapy in colorectal cancer
- Immunomodulation properties of multi-species fermented milk
- Single-strain starter experimental cheese reveals anti-inflammatory effect of *Propionibacterium freudenreichii* CIRM BIA 129 TNBS-colitis model

L. casei

Competitive influence leading to optimum 30 % SW concentration

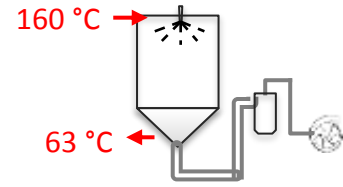
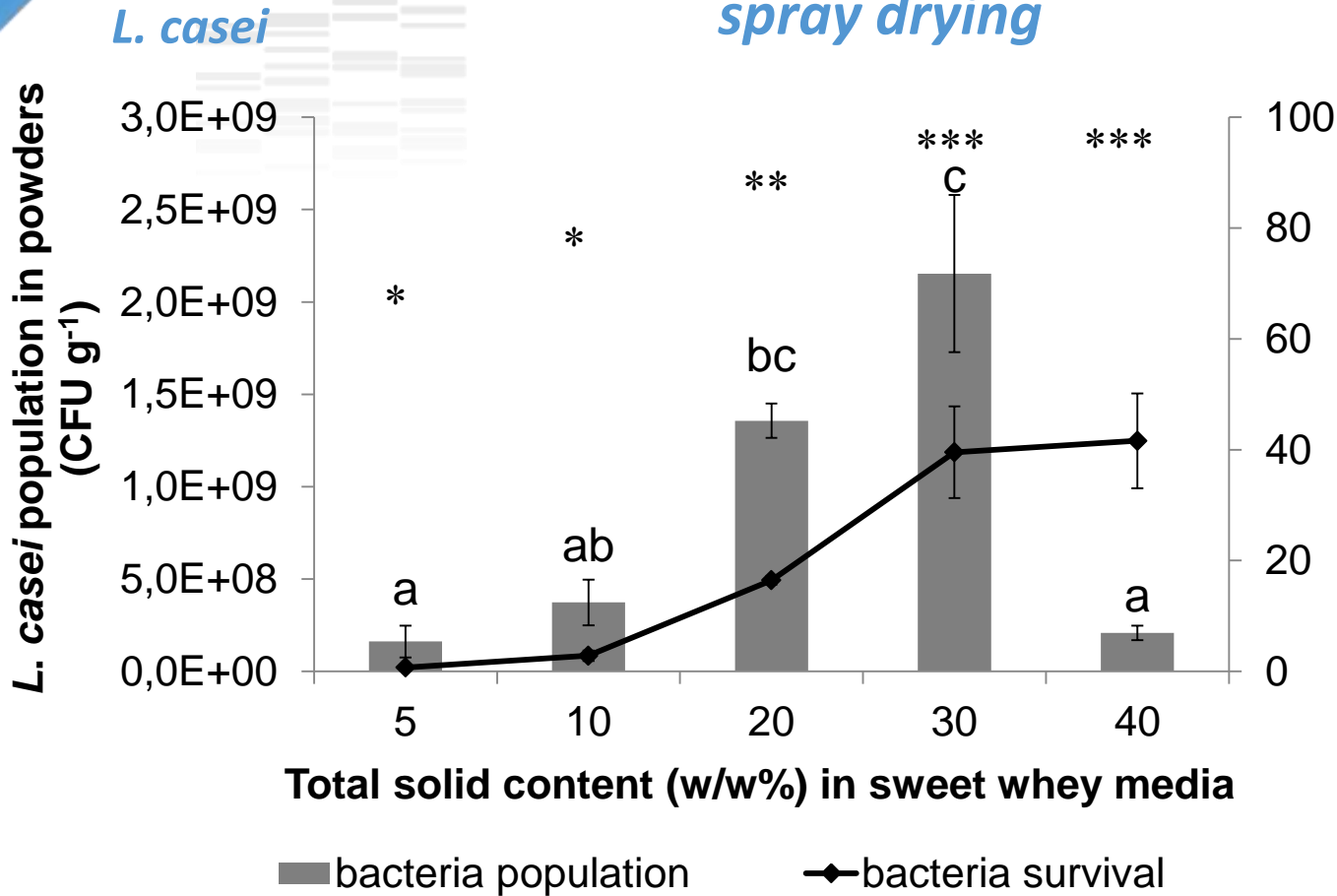
- Increasing concentration makes the nutrients (nitrogen) non-limiting
- Increasing concentration increases the osmotic pressure

2.

• A Novel Process

Bacteria behavior after spray drying

Lab-scale (~3kg/h)



Survival after spray drying (%)

30% sweet whey culture produces optimal probiotics population & survival rate

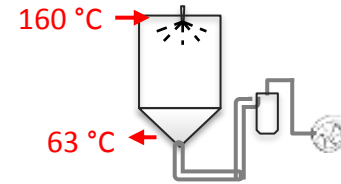
2.

• A Novel Process

A generic trend

Bacteria sp.	Optimal growth concentration	Improved post spray-drying survival
<i>P. freudenreichii</i>	20-30%	yes (→ 80%)
<i>L. acidophilus</i>	30%	yes
<i>L. rhamnosus</i>	30%	Not yet tested
<i>L. plantarum</i>	30%	Not yet tested
<i>L. reuteri</i>	20%	Not yet tested
<i>L. bulgaricus</i>	30%	Not yet tested
<i>B. infantis</i>	20%	yes
<i>B. longum</i>	20%	Not yet tested

Lab-scale
(~3kg/h)



Patented process EP 15 306465.4 - 1357

- 2-in-1 concentrated medium → simplified scheme, lower contamination risk
- higher bacteria population
- higher bacteria survival after spray drying

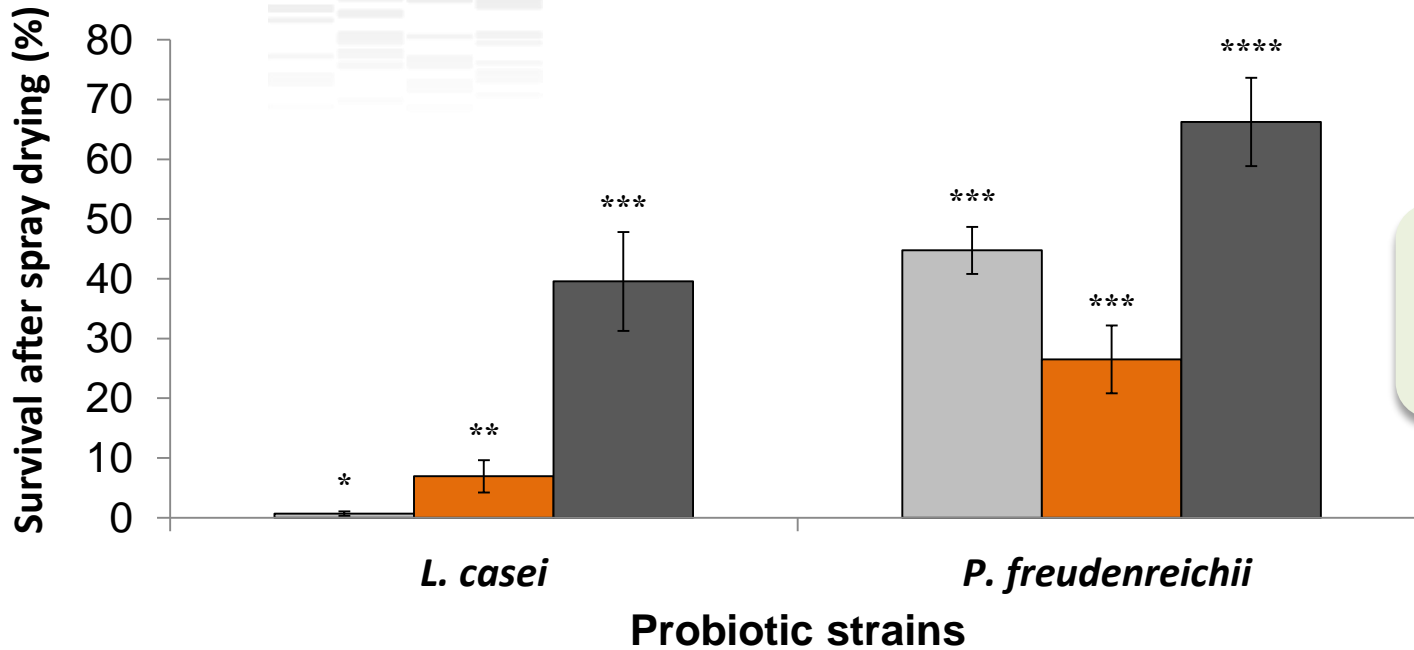


What protects probiotics?

3.

Underlying mechanisms

Is the protection due to the higher dry matter?



Growth in 30% sweet whey is the key factor

- growth with 5% TS
- growth with 5% + adding 25% TS just before drying
- growth with 30% TS

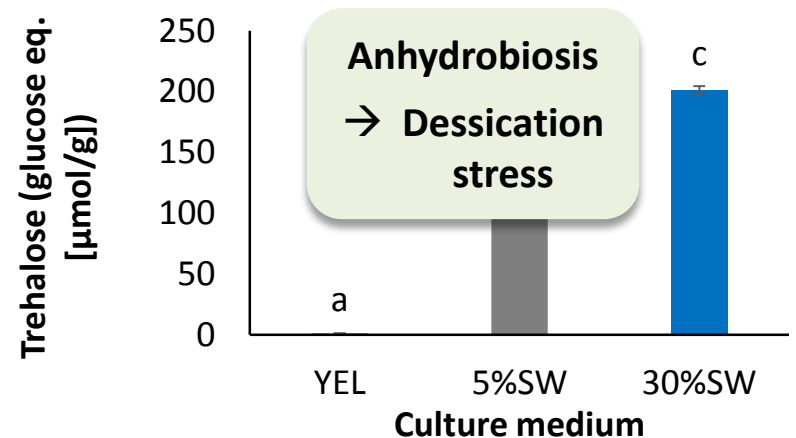
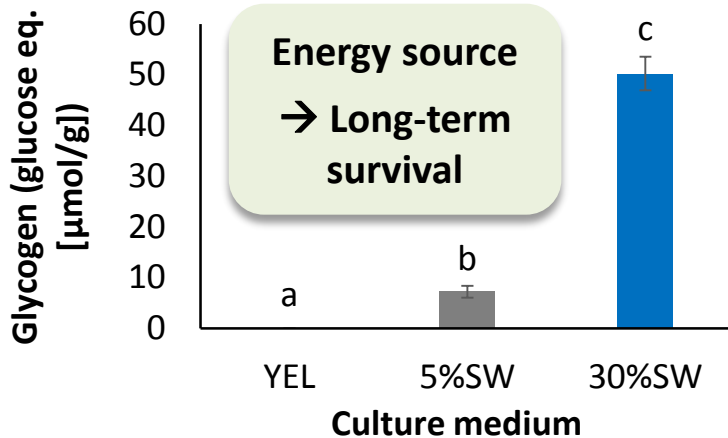
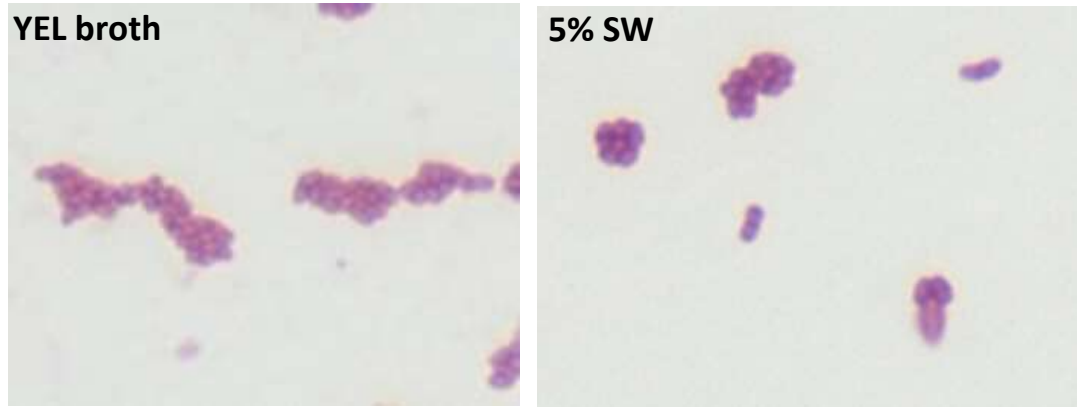
Hypothesis
30% sweet whey → Osmoregulation → Multi-stress tolerance (cross protection)

3.

Underlying mechanisms

P. freudenreichii

Periodic acid-Schiff staining



30% sweet whey induces bacterial sugar accumulation for osmoregulation

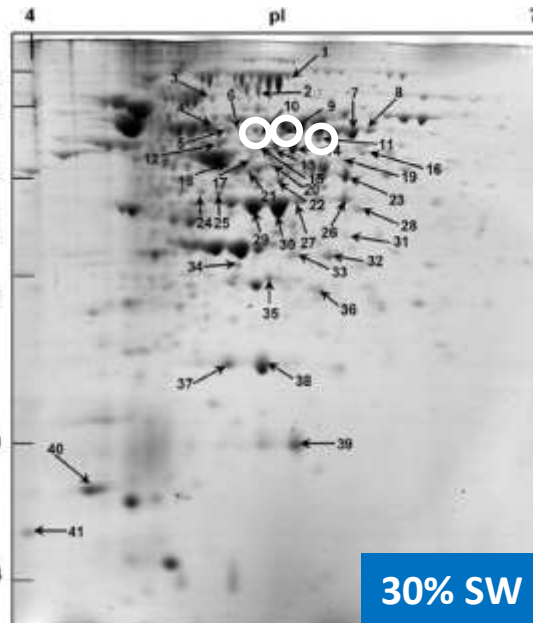
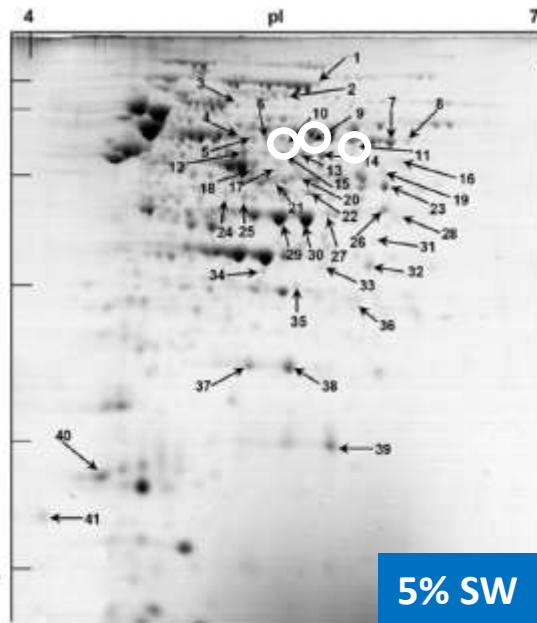
3.

Underlying mechanisms

P. freudenreichii

Osmoregulation during growth - Protein upregulation

2-D gel proteomic analysis



Osmoregulation proteins

- **Glycine betaine transporter**
ATP-binding OpuCA of osmoprotectant ABC transporter
- **Choline synthesis**
Glycine hydroxymethyltransferase
- **Proline synthesis**
Xaa-Pro aminopeptidase

30% sweet whey upregulates bacterial osmoregulation proteins
→ osmoregulation

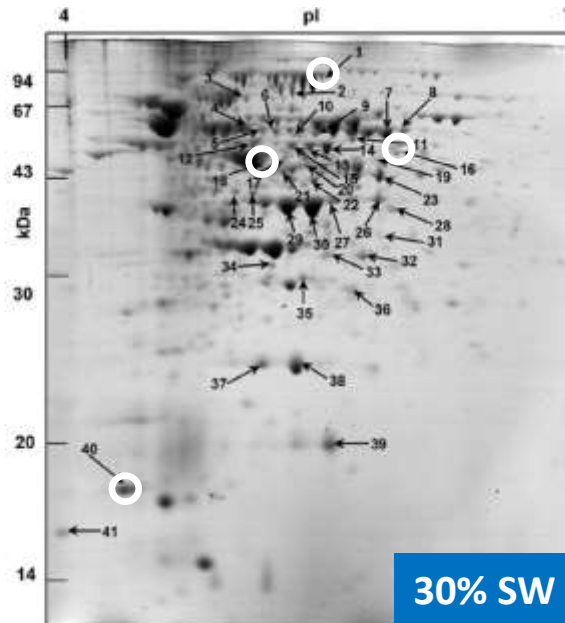
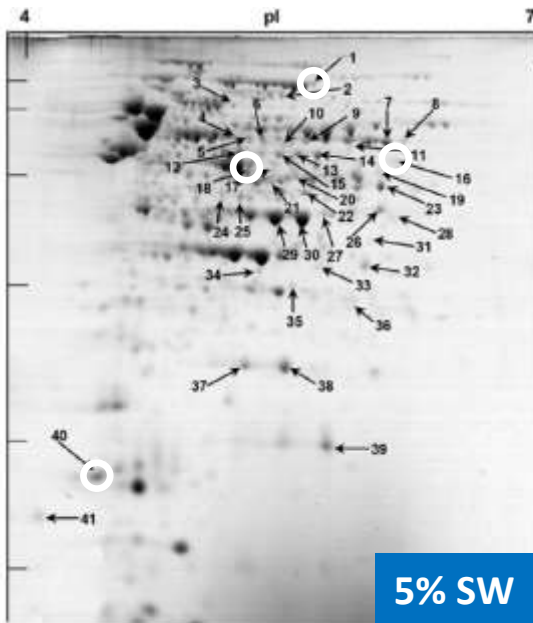
3.

Underlying mechanisms

P. freudenreichii

Osmoregulation during growth - Protein upregulation

2-D gel proteomic analysis



Stress proteins

- **Multi-stress tolerance**
ClpB 2 chaperone
Elongation factor EF-Tu
- **Heat tolerance**
Heat shock protein 20
- **Oxidative tolerance**
Cysteine synthase

30% sweet whey upregulates bacterial multi-stress proteins
→ cross protection

3.

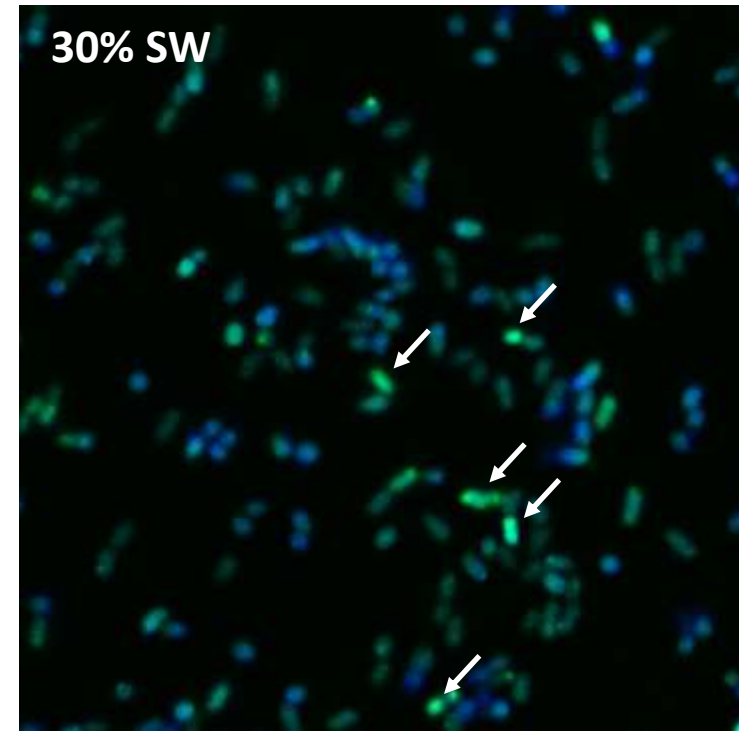
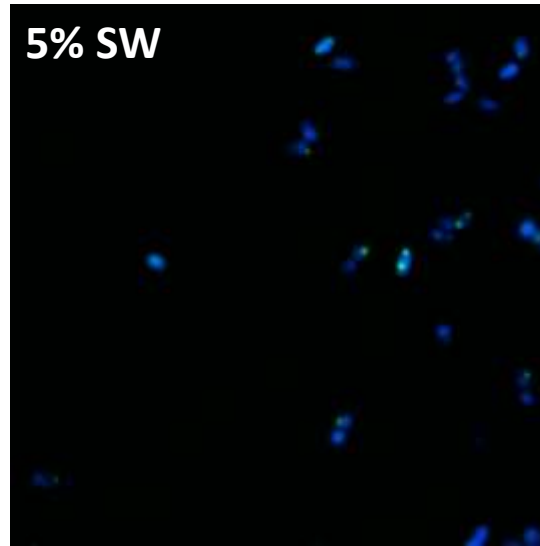
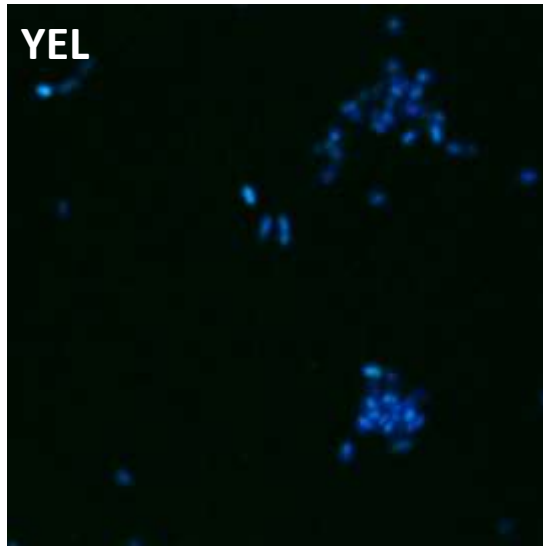
• Underlying mechanisms

P. freudenreichii

DAPI staining, confocal microscopy

- Green fluorescence indicates poly(P)
- Blue indicates DNA

Cross protection acquired during growth - Accumulation of polyphosphate



30% sweet whey induces bacterial polyP accumulation

→ cross protection / ATP homeostasis and repairing of damaged proteins

Current Opinion in Microbiology, 2015, 24:1–6

3.

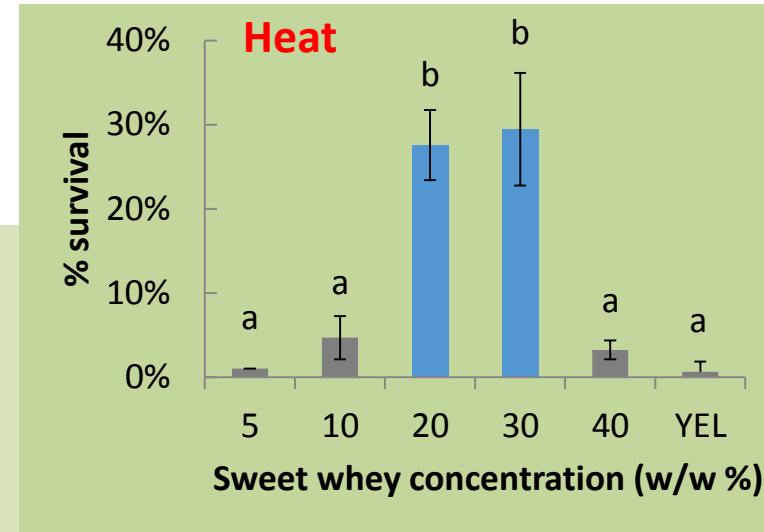
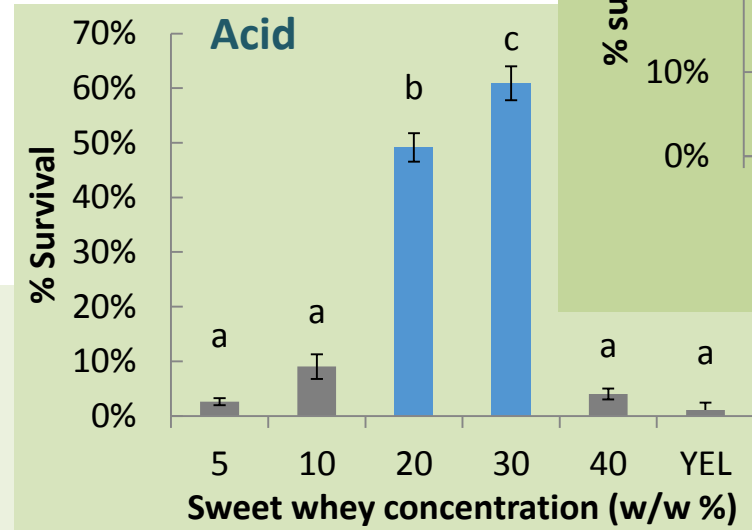
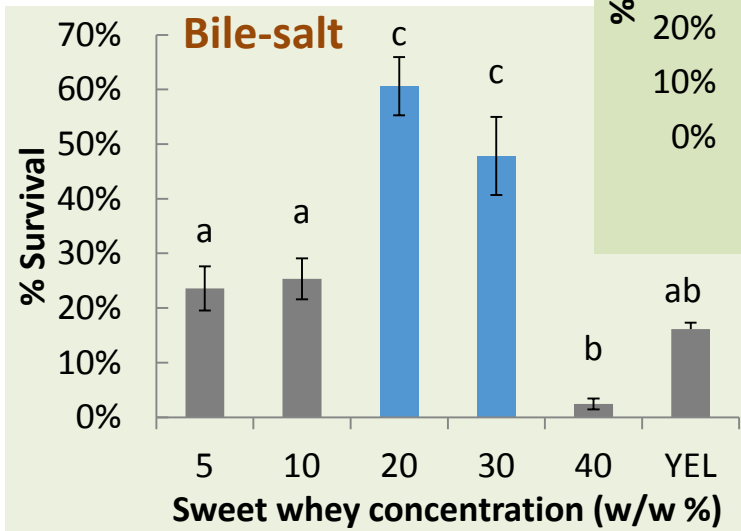
Underlying mechanisms

P. freudenreichii



Bacteria stress challenge

Resistant phenotype of bacteria?



Bacteria in 20~30% sweet whey are multi-stress tolerant

3.

Underlying mechanisms

L. casei



Growth



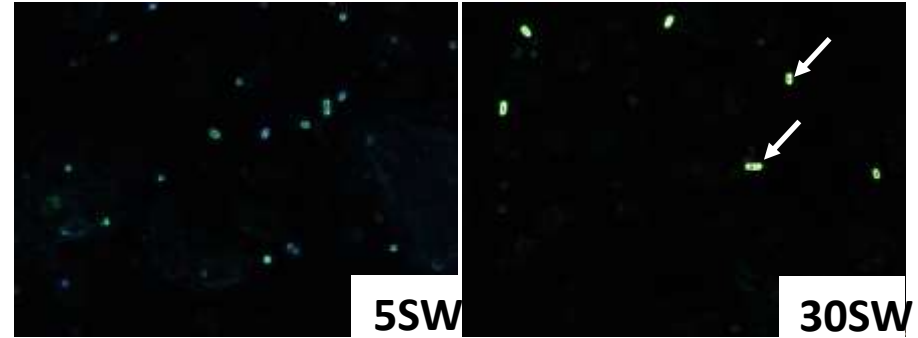
30 SW



Protein upregulation
(Label-free proteomics):

- Osmoregulation
- Multistress tolerance

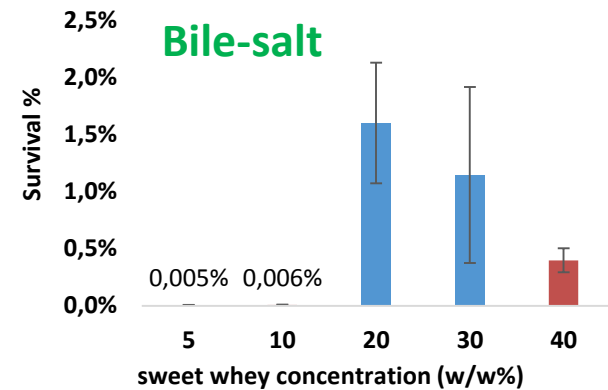
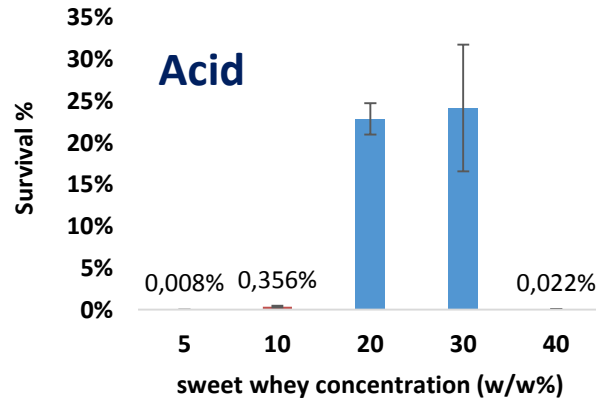
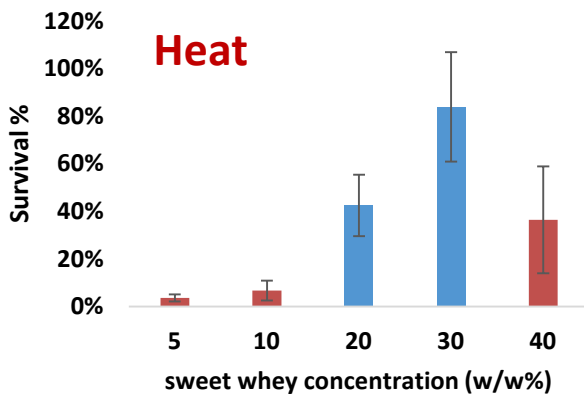
Generic osmoregulation induced protection mechanisms



Polyphosphate accumulation

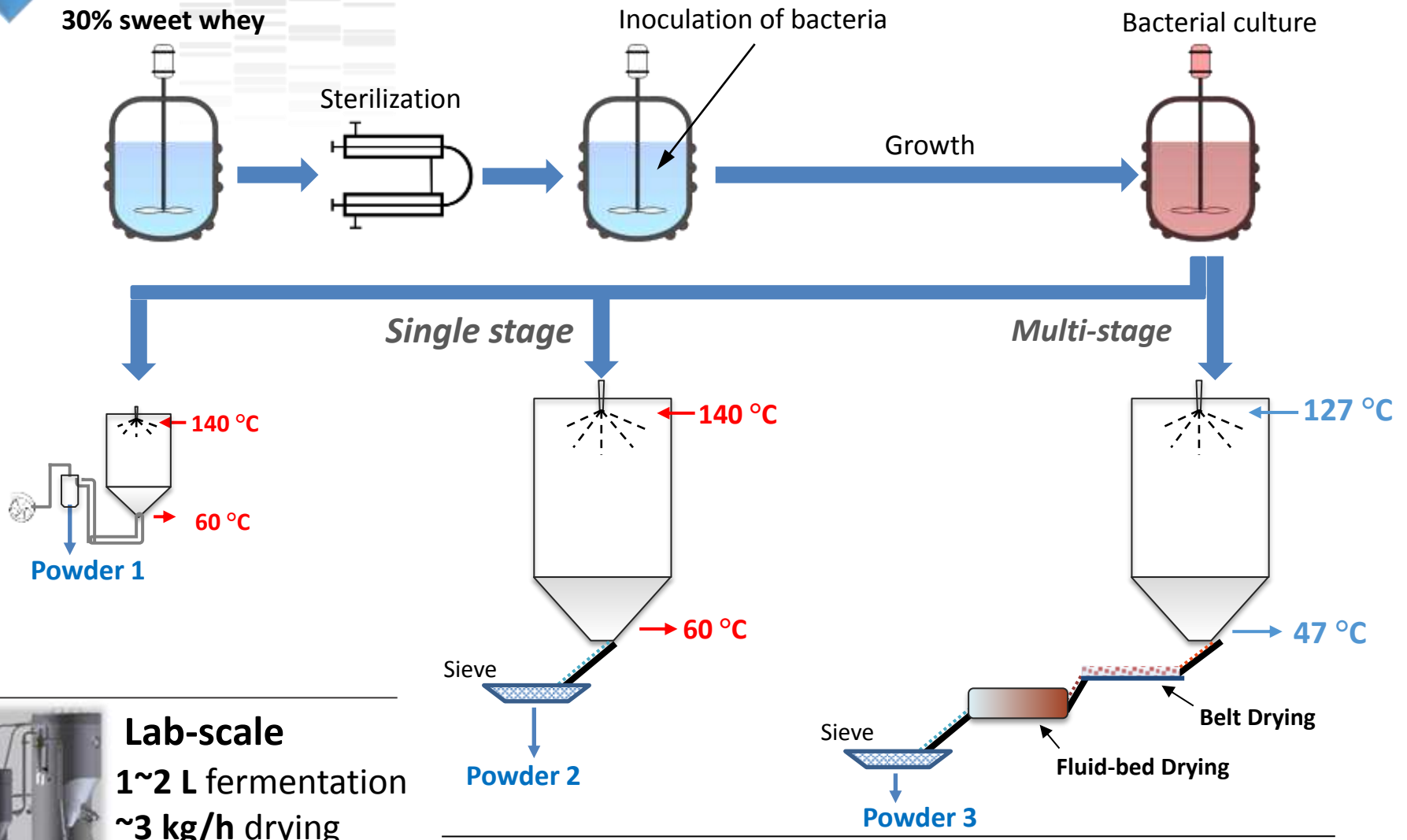


Multi-stress tolerance



4.

Scale-up and application



Lab-scale
 1~2 L fermentation
 ~3 kg/h drying

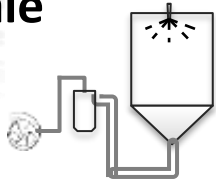


Pilot-scale BIONOV
 500 L fermentation
 ~100 kg/h drying

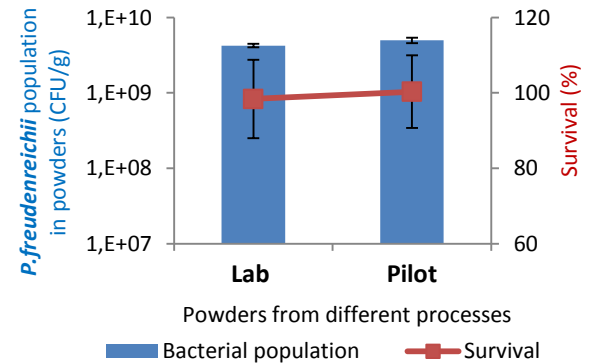
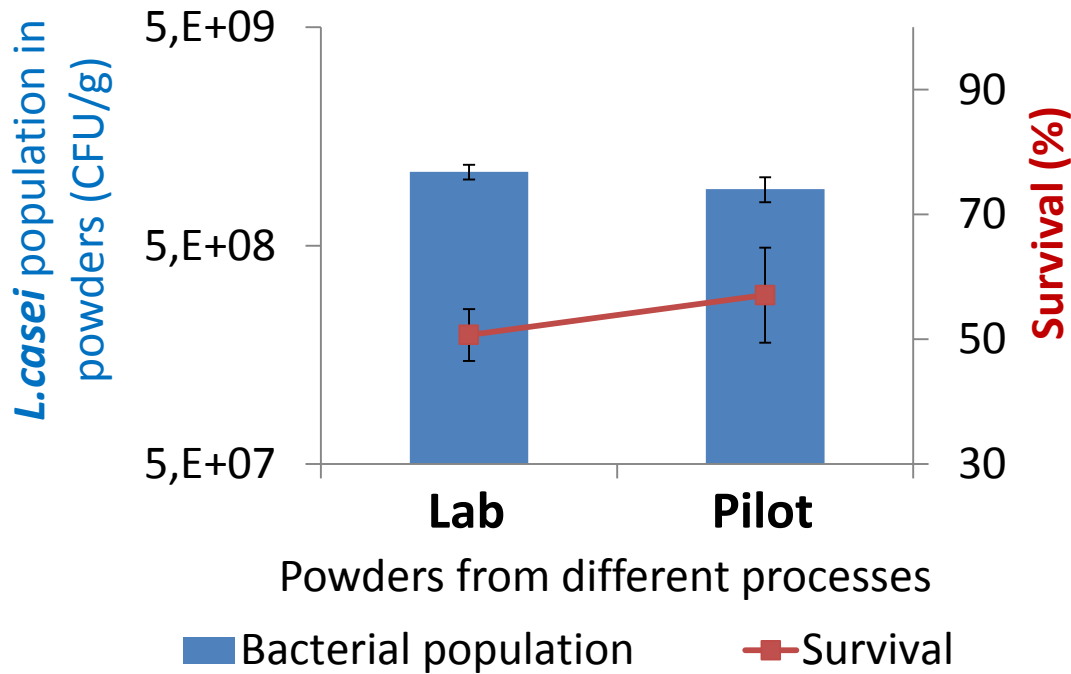
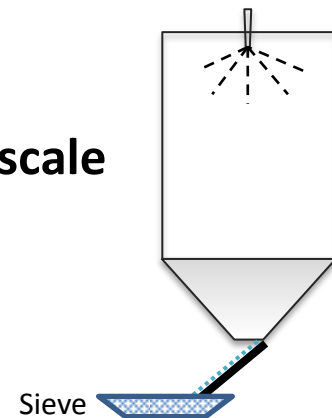
4.

• Scale-up and application

Lab-scale



Pilot-scale

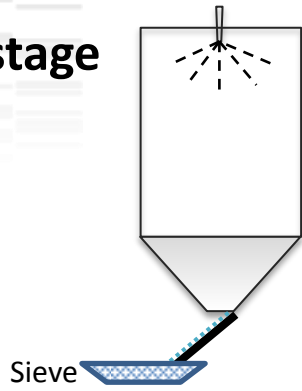


Scale-up feasibility

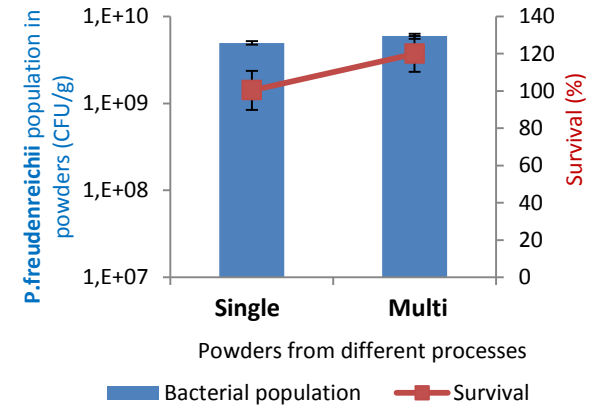
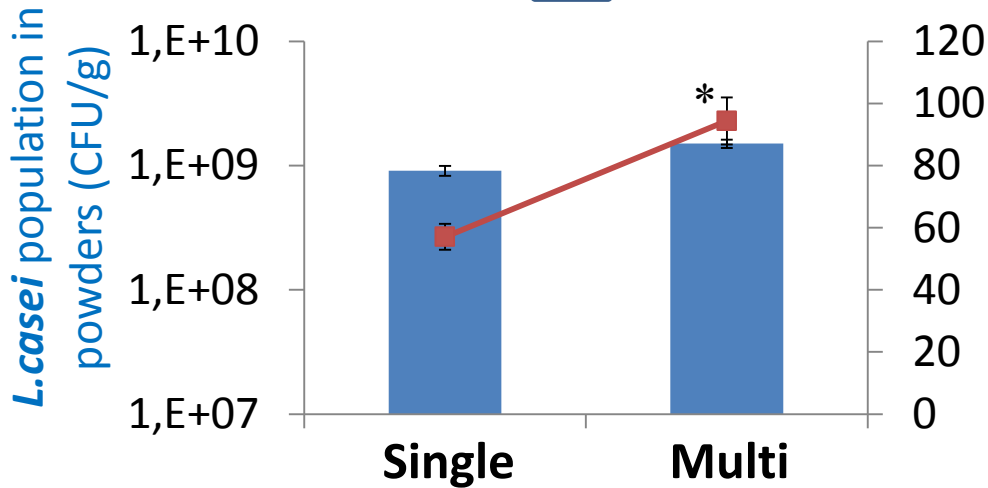
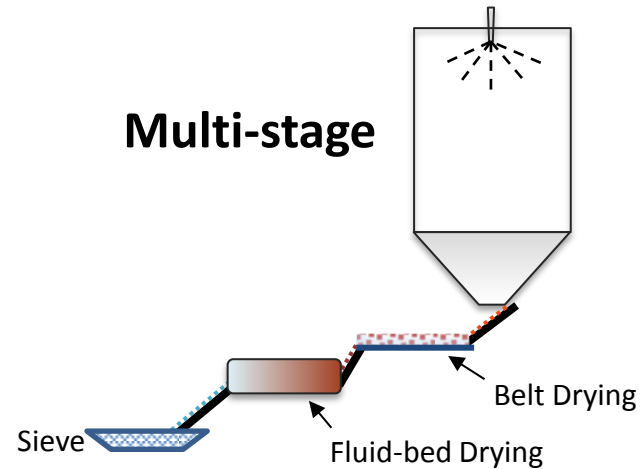
4.

• Scale-up and application

Single stage



Multi-stage



Powders from different processes

■ Bacterial population ■ Survival

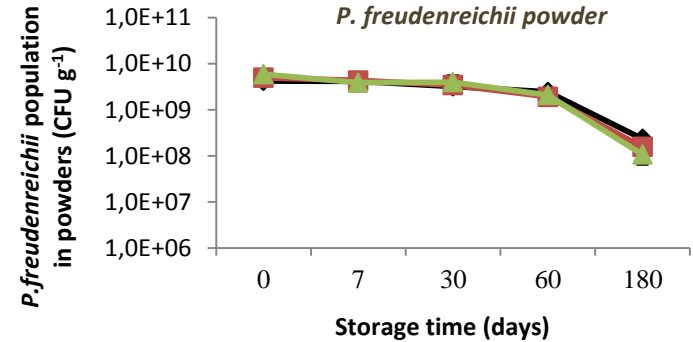
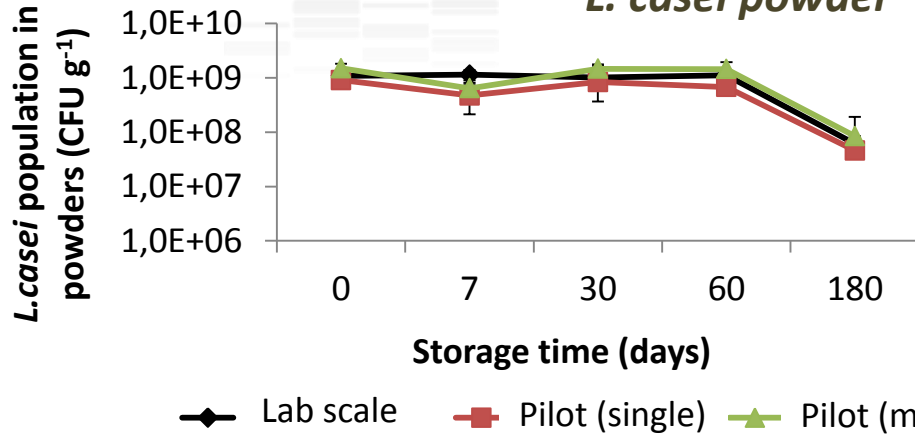
2-in-1 process run with multi stage scheme provides 100% survival

• Scale-up and application

Storage and digestion stability

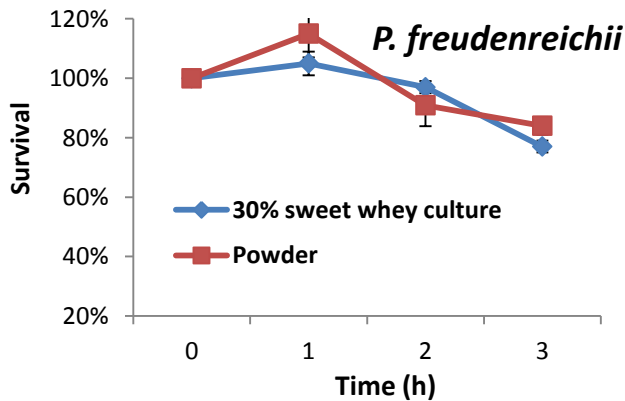
Storage at 4°C

L. casei powder

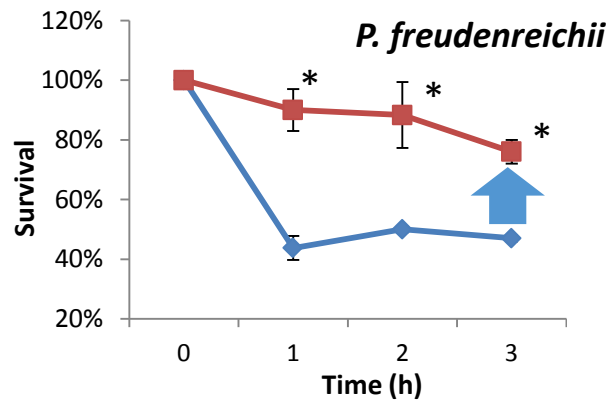


In vitro digestion behavior: Fresh culture vs. Rehydrated powder

Simulated gastric fluid



Simulated intestinal fluid



- Culture ≈ Powder in gastric survival
- Induced intestinal resistance, depending on the specie

One step, food grade, metabolites, energy savings...



Microbiology

Osmotic

Multistress tolerance



Material

Concentrated whey

Mineral salts (Mg^{2+})
Benefit of aggregates



Processing

Pre-concentration

Multi-stage drying

- ✓ 100% survival
- ✓ Scale up feasibility
- ✓ Storage & Digestion behavior

In vivo assessment

24 piglets
(*P. freudenreichii*)

Fresh probiotic culture
Sweet whey powders
2-in-1 probiotic powders

controls

Work in Progress

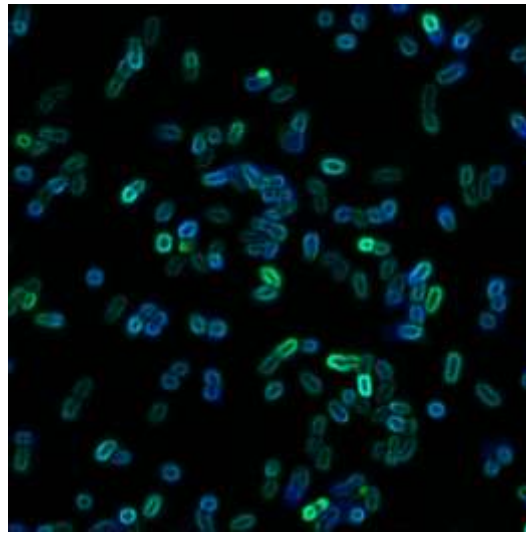
Functional components in GIT and in feces

- Distribution of *P. freudenreichii*
- Immunomodulatory S-layer proteins content
- Short Chain Fatty Acids content

Do the gut associated immune cells have a significantly different activity ? (*Peripheral blood mononuclear cells , Immune cells from mesenteric lymph nodes*)

→ Immunomodulation markers : Cytokines (IFN γ , TNF α , IL-8, IL-10) after stimulation by anti & pro- inflammatory stimuli

Merci



Thank you

for your attention... Any questions?

More information

- Huang et al., 2016, *J Funct Foods* **23**, 455-463
- Huang et al., 2016. *Applied Env Microbiol* **82**, 4641-4651
- Zheng et al., 2016. *Food Res Int* **90**, 226-234
- Yang et al., 2017. *Lett Appl Microbiol* **64**, 283-288
- Huang et al., 2017, *J Food Eng* **196**, 11-17
- Huang et al., 2017, *Trends Food Sci Technol* **63**, 1-17