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Center for Microbial Ecology and Technology - Faculty of Bioscience Engineering - Ghent University

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Probiotics delivery: does the matrix matter?

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Pre- & Probiotics in Paediatrics

April 28th, 2016

Gent (Belgium)





Introduction

Functional stability



Hours



Days

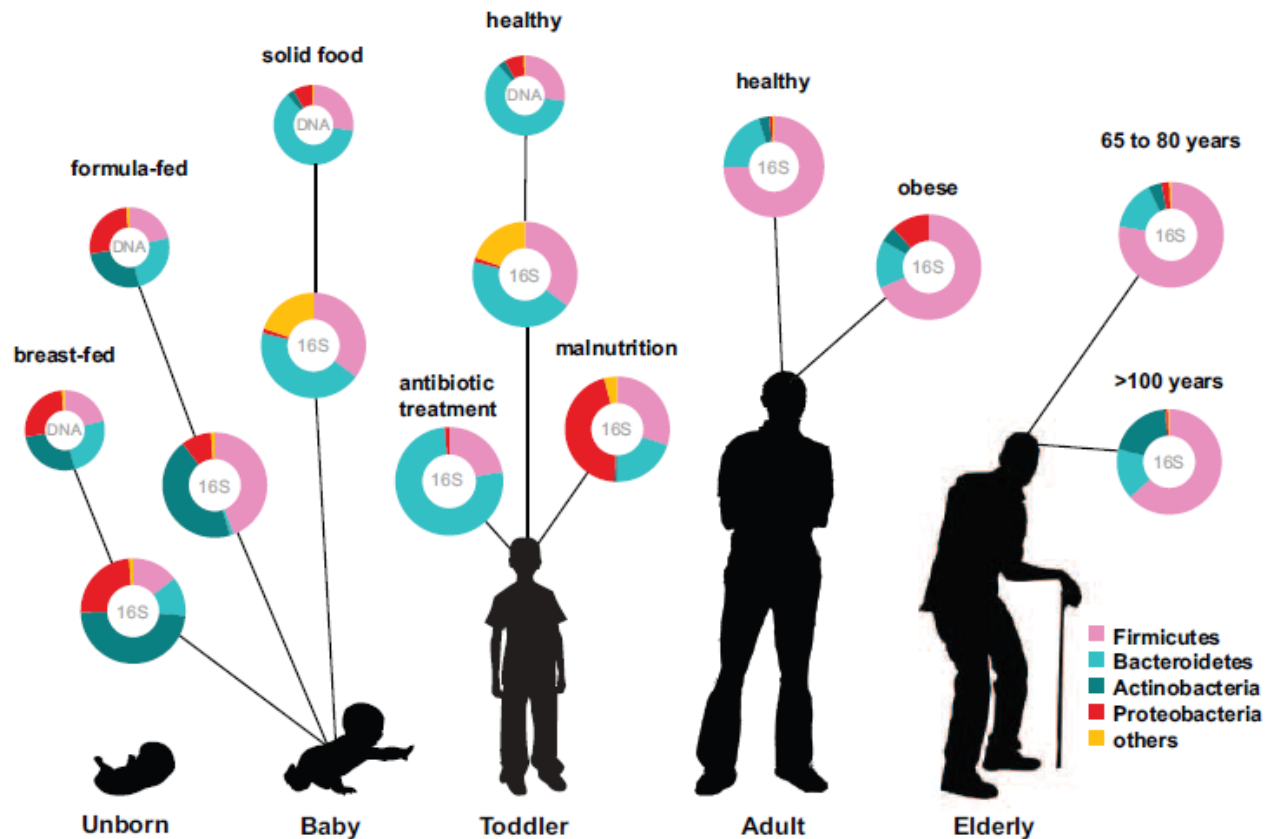


Months





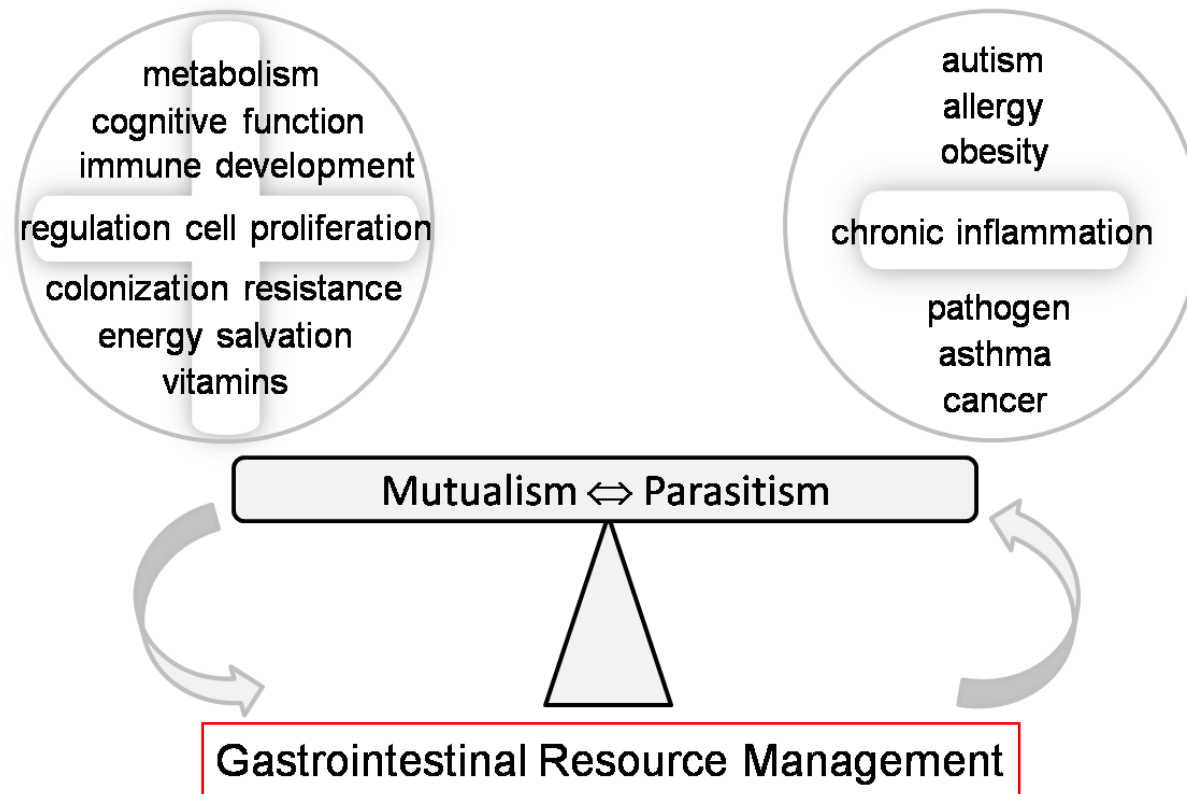
Introduction





Introduction

Asset/Liability





Introduction

- Several factors concur in shaping the gut microbiota
 - Delivery method (natural vs. cesarean section) brings to a different cross-contamination
 - Environment
 - Family habits
 - Geography, climate
 - Genetics
 - Diet (breast feeding vs. formula-fed)

Fecal microbiota	
<i>Breast-fed</i>	<i>Formula-fed</i>
↑ Bifidobacteria	↓ Bifidobacteria
↑ Lactobacilli	↑ Bacteroides
↑ Gram ⁺ cocci	↑ Coliforms



Introduction

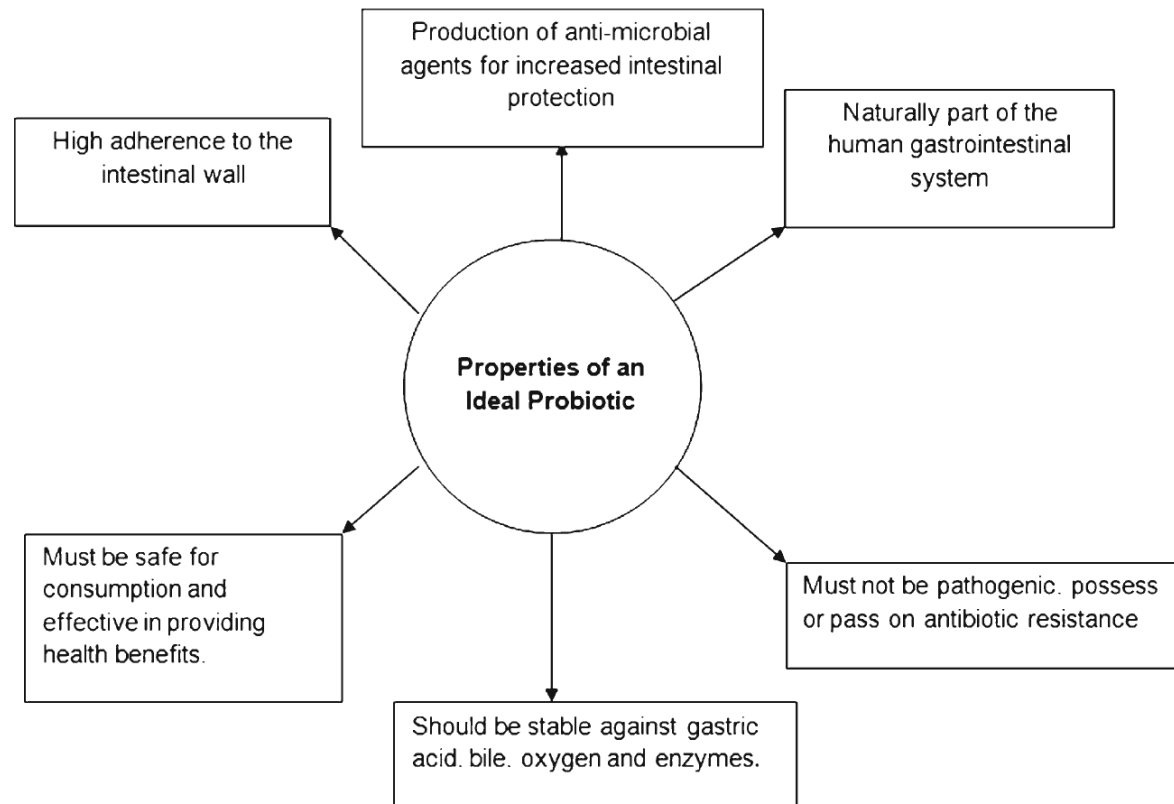
- **Mother's milk** composition represents a continuous supply of commensal, mutualistic and/or potentially **probiotic bacteria** to the infant gut and a **unique mixture of oligosaccharides** that change in composition during the first months of life of the baby
- Breast feeding is not always possible
- Great interest in identifying alternatives
- **Pre- and probiotics added to baby formula**





Probiotic

- Probiotics can be defined as **live microorganisms** which when dosed in adequate amounts **confer a health benefit** on the host





Probiotic

- Two crucial aspects determine the success of a probiotic treatment:
 - The **resistance** of the bacteria to production, storage and the harsh conditions of the **upper intestine**
 - The capacity to **compete with the indigenous intestinal bacteria** in the colon.
- **Intrinsic characteristics of the probiotic strains** (e.g. acid and bile tolerance)
- **Specific formulation** in which they are delivered to the gut (higher protection = more efficient competition with the resident microbial community)



Two main questions

- How can we study the survival of probiotic strains in areas of the gut that are not easily accessible?
- What is the potential role - if any - of the delivery matrix or technology?



Research methods

- Human intervention studies
- *In vitro* simulation technologies
 - Advantages:
 - Easier setup and sampling
 - High reproducibility
 - Mechanistic studies possible
 - Representative to a specific process
 - No ethical constraints
 - Medium to high throughput
 - Disadvantages:
 - Absence of physiological environment
 - Human studies are necessary for confirmation





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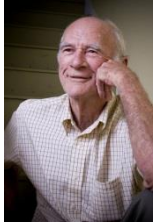
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ProDigest
Gastrointestinal Expertise



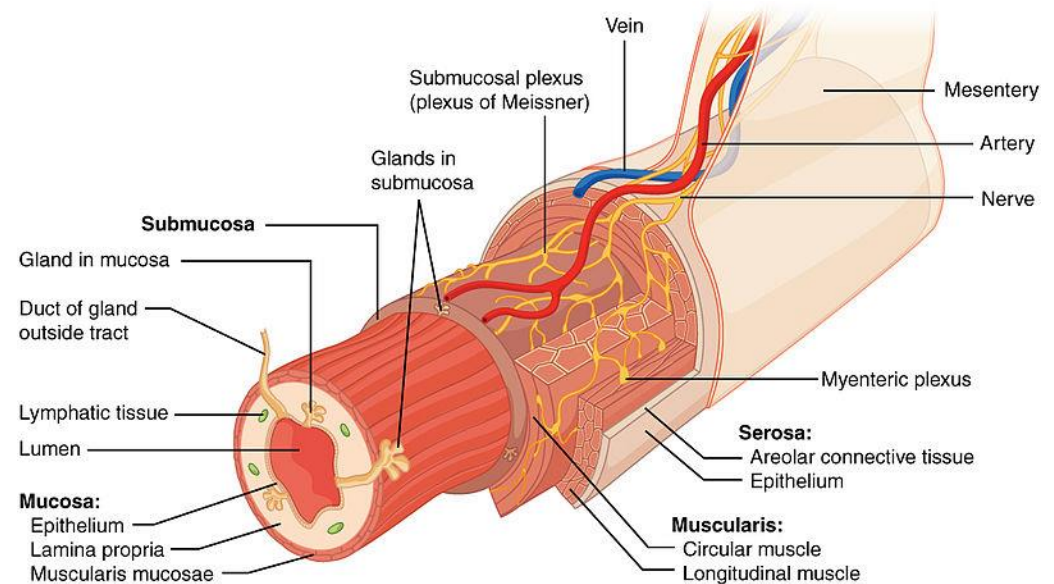
SHIME[®] technology platform



SHIME[®]: Simulator of the Human Intestinal Microbial Ecosystem

SHIME[®] technology platform

The better an *in vitro* system can simulate the real gut situation, the higher is the physiological significance of the obtained information



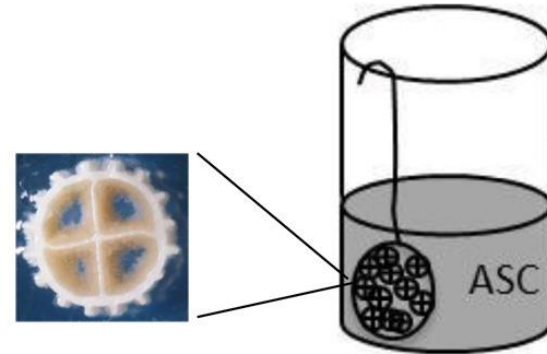
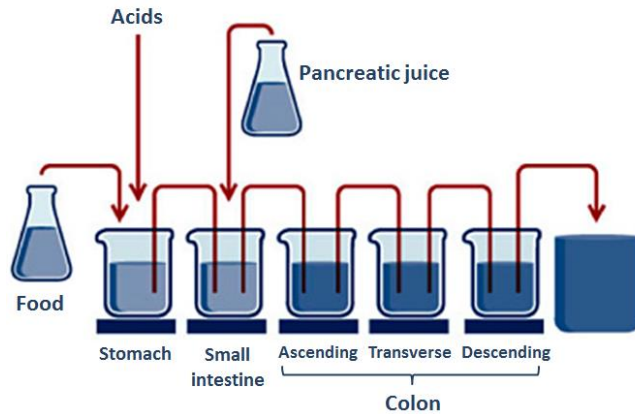
What about the gut wall?

- Mucus layer
- Host simulation



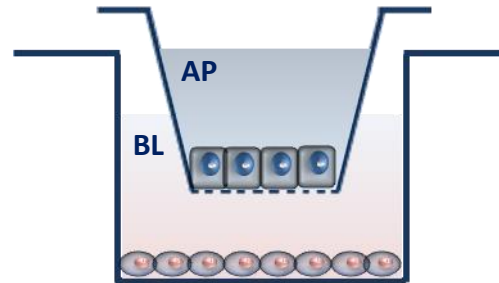
SHIME® technology platform

Introducing the “host-compartment”



Intestinal cells: Caco-2
monolayer
(cellular model for
intestinal epithelium)

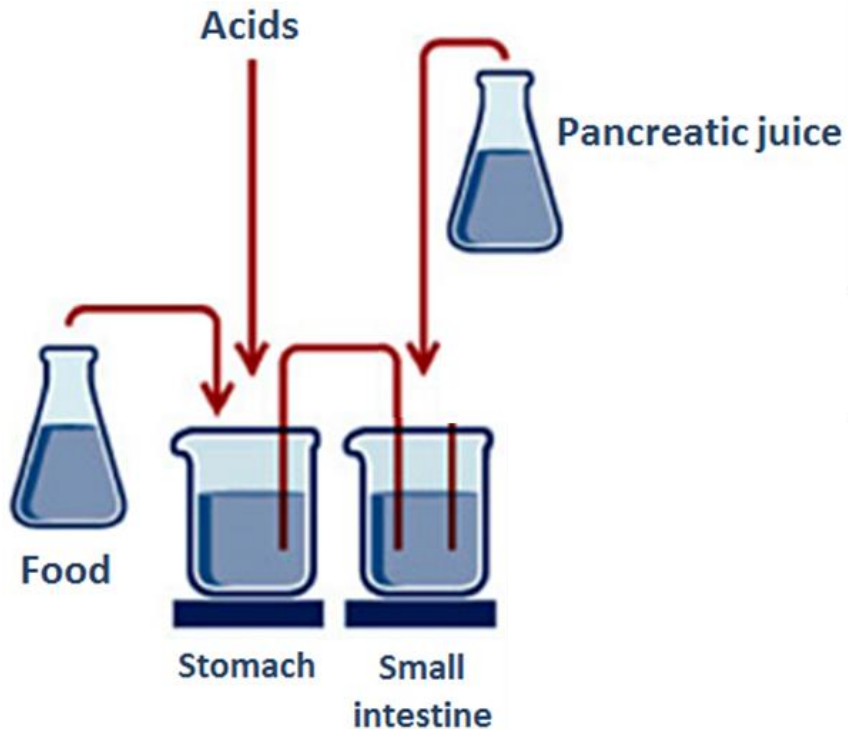
Immune cells: activated THP1
macrophage-like cells (PMA)]





Delivery of probiotics

Fasted vs. Fed



Fasted

- Quick transition
- Low pH in the stomach
- Low bile salts and pancreatic juices in the small intestine

Fed

- Longer transition
- Sigmoidal decrease of the pH in the stomach
- High bile salts and pancreatic juices



Delivery of probiotics

❖ Encapsulation technology

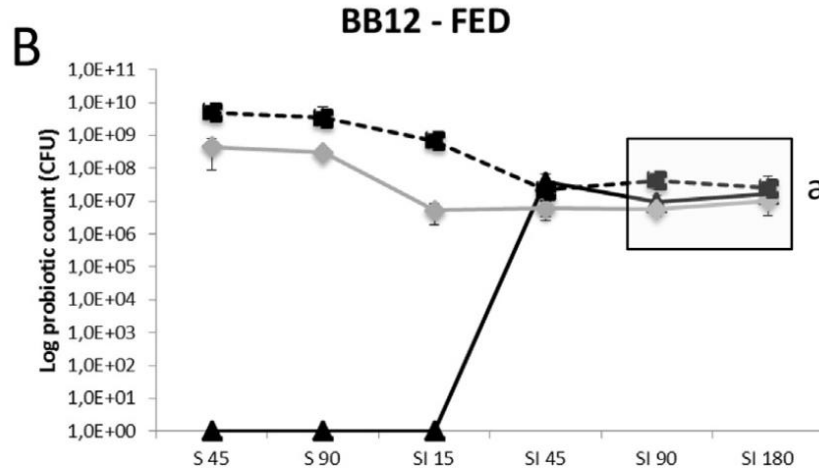
- Vcaps[®], Vcaps[®] Plus and DRcaps[™] (hypromellose capsules from Capsugel)
- Coni-Snap[®] Hard Gelatin sprinkle Capsules (Capsugel)
- Microencapsulation technology - Intelicaps[®] (Vesale Pharma)

❖ Food matrix

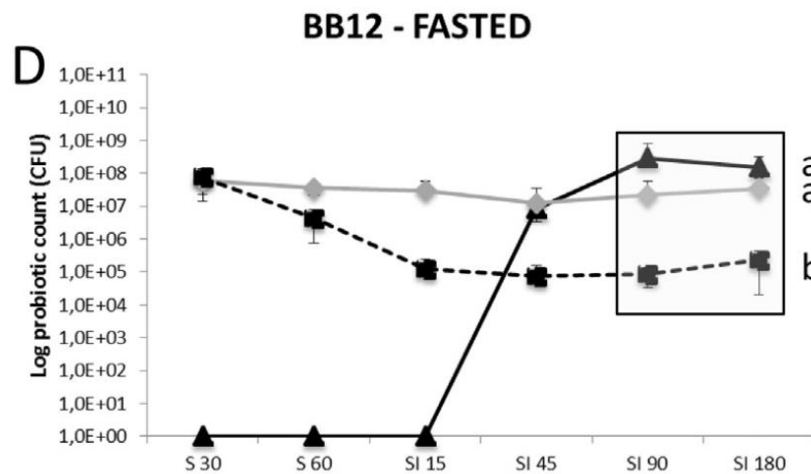
- Fermented milk
- Chocolate

Delivery of probiotics 1

Black triangle = DRcaps™; gray diamond = Vcaps®; black square = Vcaps® Plus



Effect of bile salts



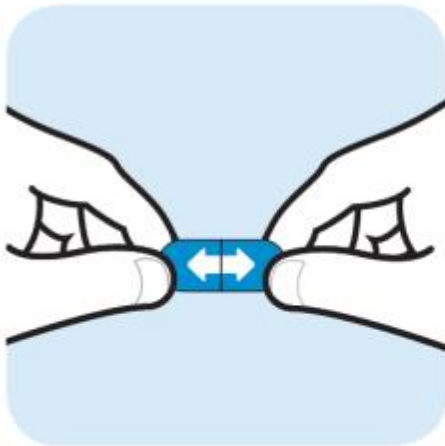
Protection from pH



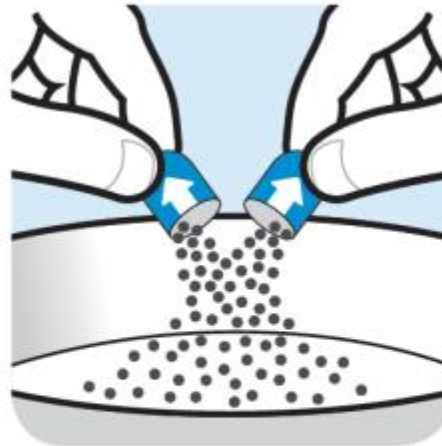
Delivery of probiotics 1

The technique applied for the delivery of the probiotic plays a role on the **strain survival** -> the preferential approach should also take into **account the end user**

Coni-Snap® sprinkle capsules



Instructional arrows indicate opening of capsule.



Easily opened for sprinkling onto soft food.



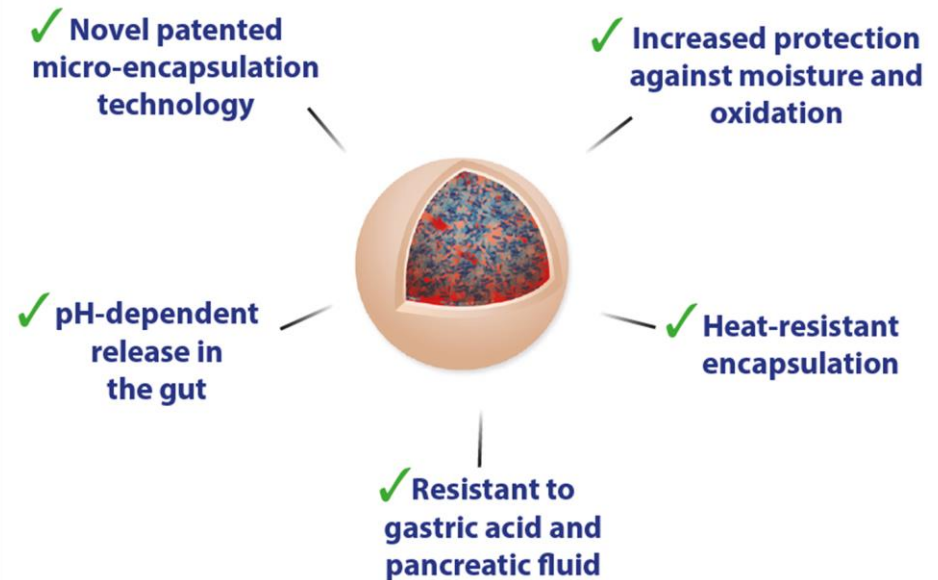
Contents mixed with soft food for oral administration.



Delivery of probiotics 2

Use of **microencapsulation (Intellicaps®)** to protect 2 strains of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp. *lactis*

White spherical, uniform particles (size 150 – 600 µm)



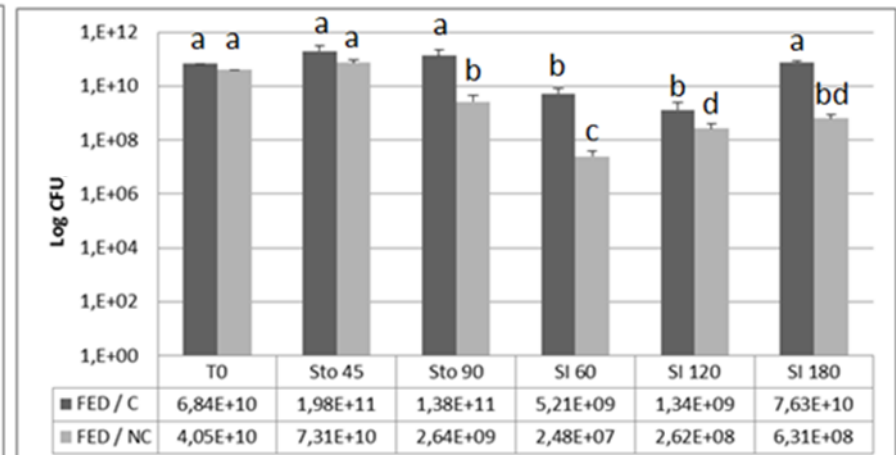
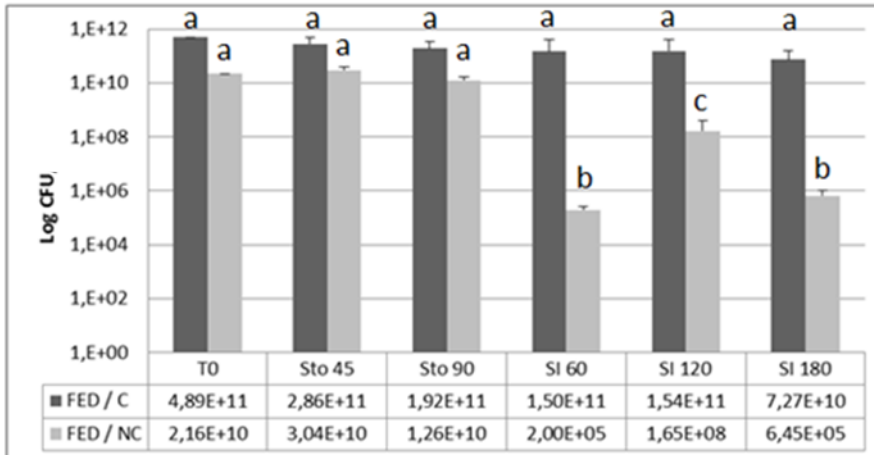


Delivery of probiotics 2

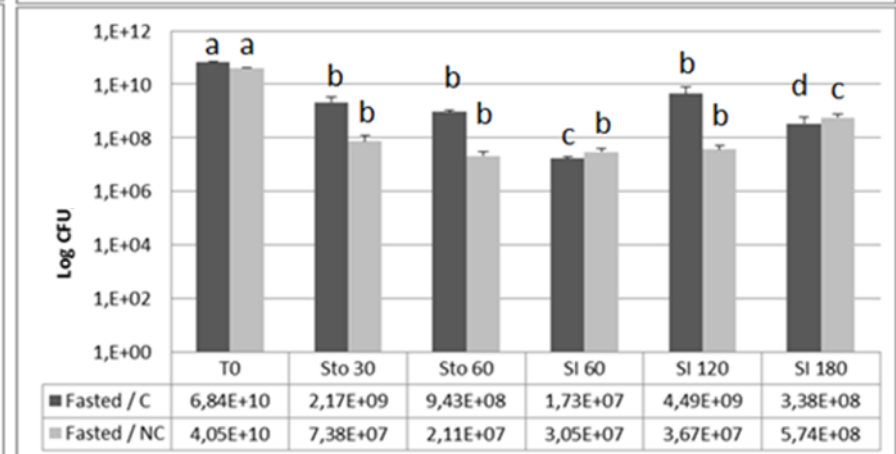
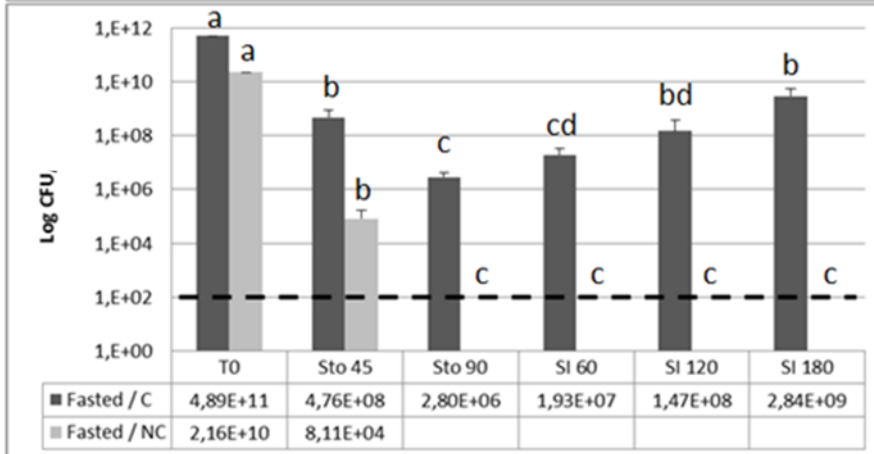
L. rhamnosus

B. animalis subsp. lactis

Fed



Fasted



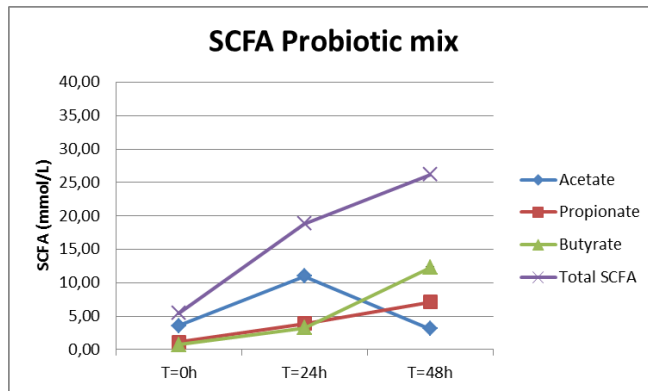
Encapsulation improved the viability of the probiotic strains



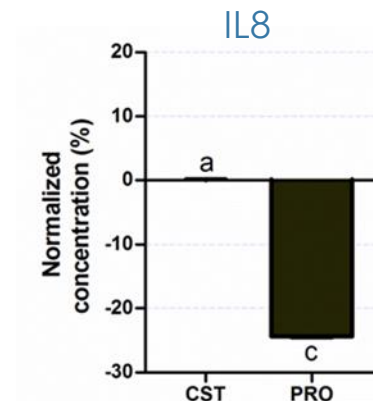
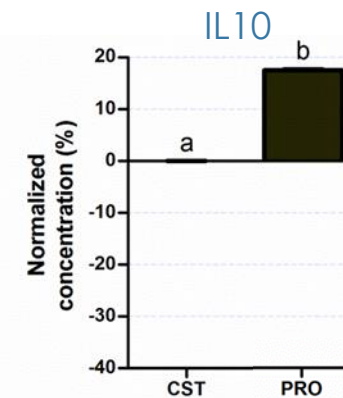
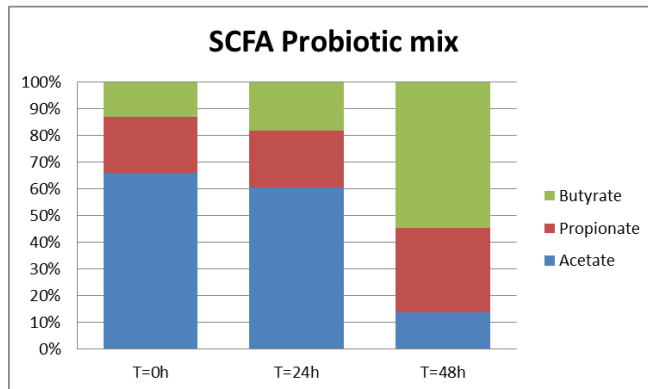
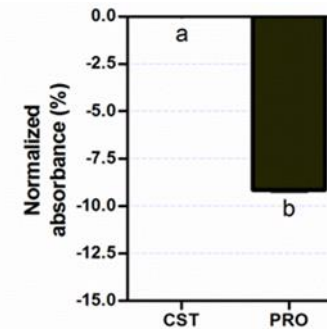
Delivery of probiotics 2

Gut metabolism proximal colon

Effect on the host



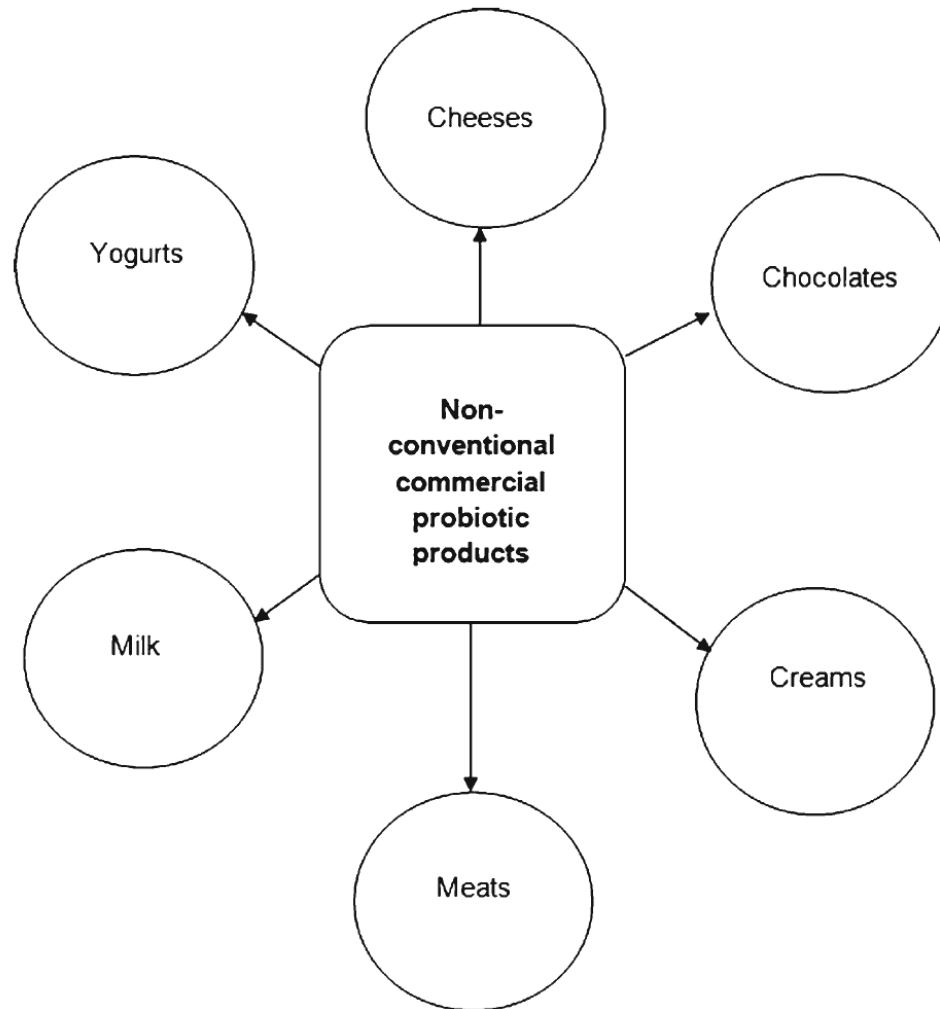
NF- κ B – AP1 activity



Improved SCFA production in the proximal colon and anti-inflammatory activity



Delivery of probiotics 3

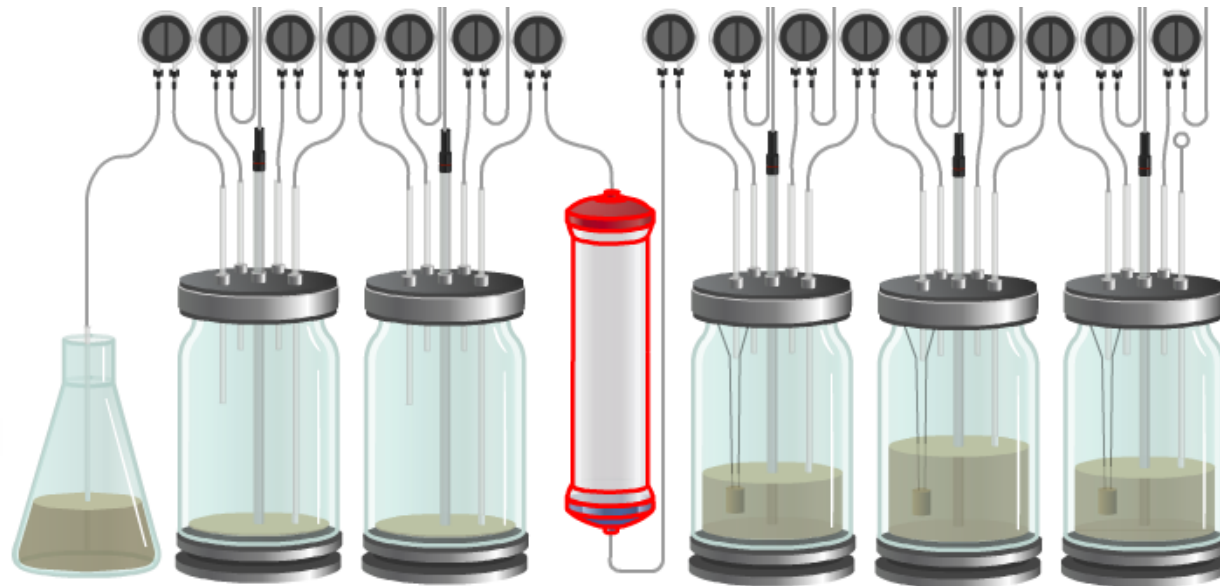




Delivery of probiotics 3

Simulation of small intestinal absorption via dialysis

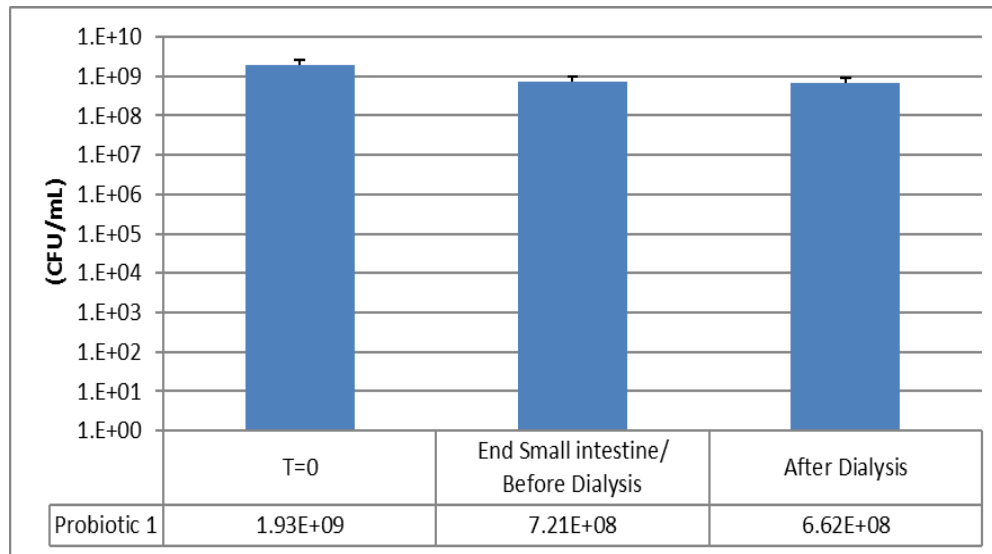
- Case study: Probiotic yoghurt
 - Probiotic needs to reach the colon in good conditions
 - However, yoghurt matrix needs to be digested and absorbed before entering the colon



SHIME® technology with absorption modeling

Delivery of probiotics 3

Probiotic bacteria out:
(7,2 ± 1,6) x 10⁸ CFU/mL



Probiotic bacteria in:
(6,6 ± 1,3) x 10⁸ CFU/mL

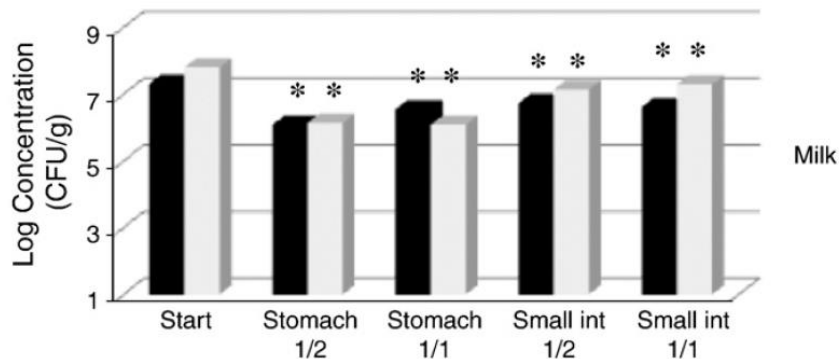
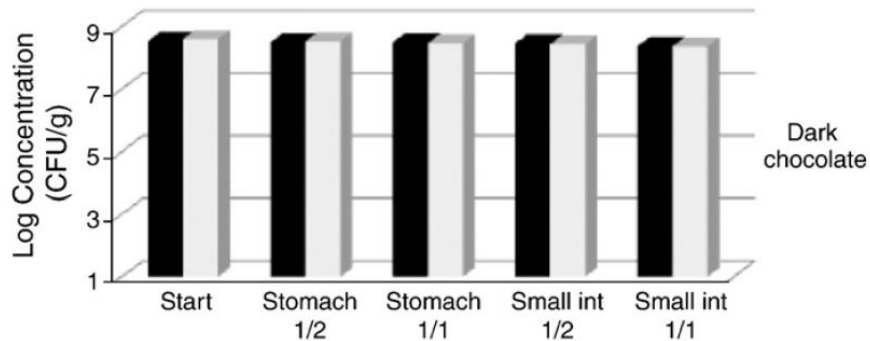
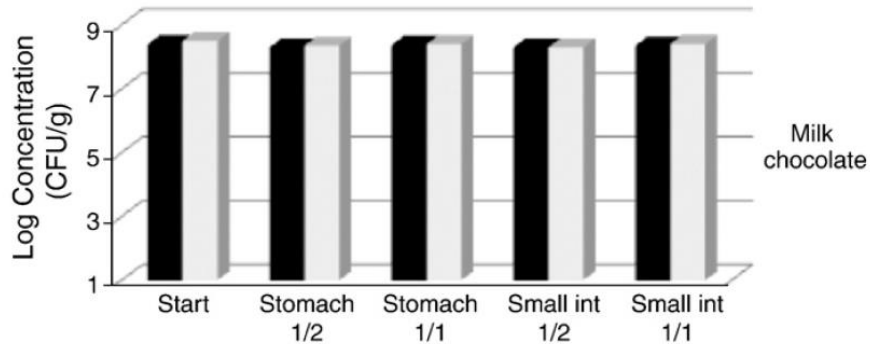
62 mg yoghurt N out



**81% yoghurt
N removal**

324 mg yoghurt N in

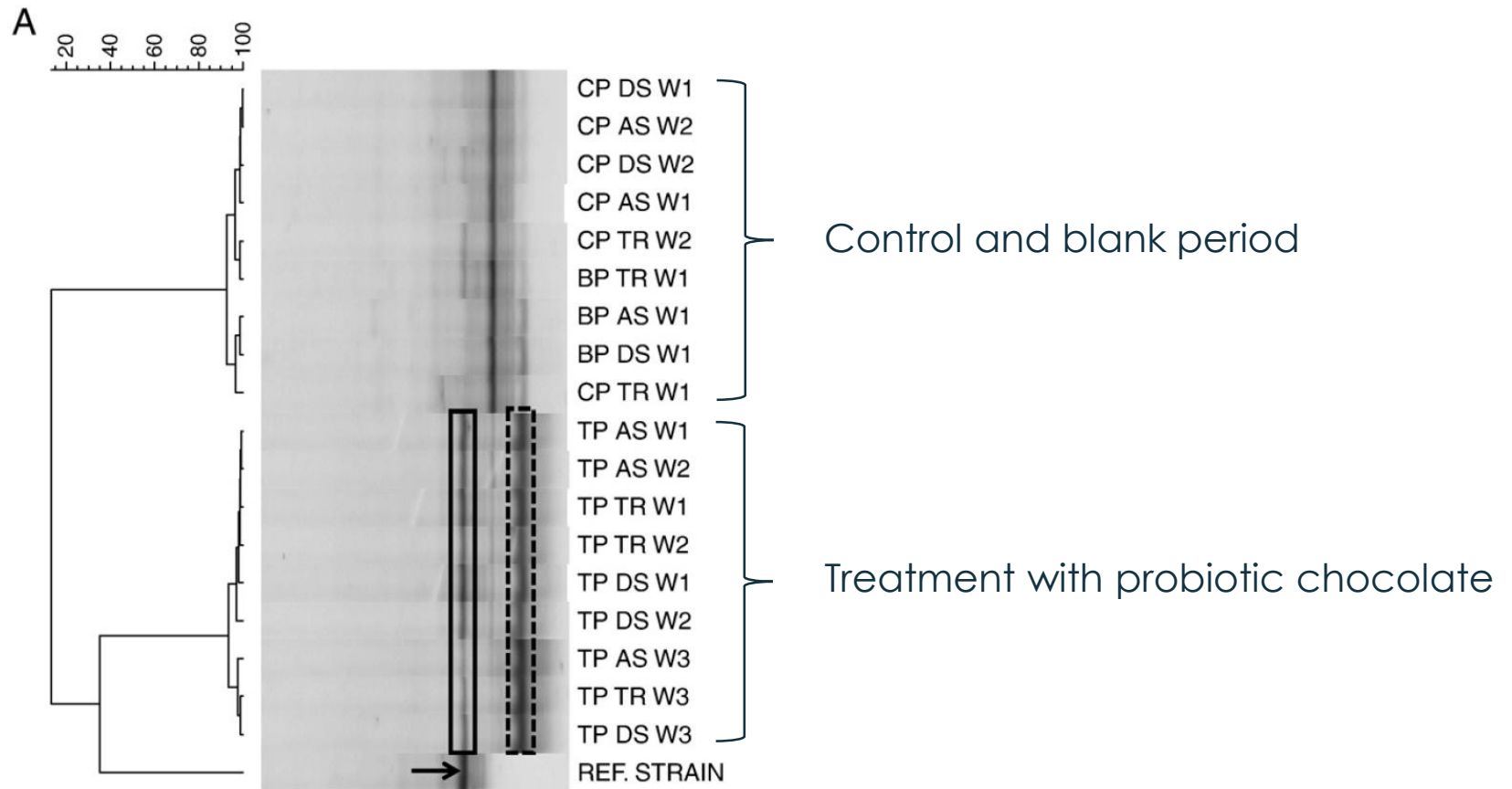
Delivery of probiotics 4



Survival of *L. helveticus* CNCM I-1722 (black bars) and *B. longum* CNCM I-3470 (grey bars) embedded in a dark or milk chocolate or milk matrix

Delivery of probiotics 4

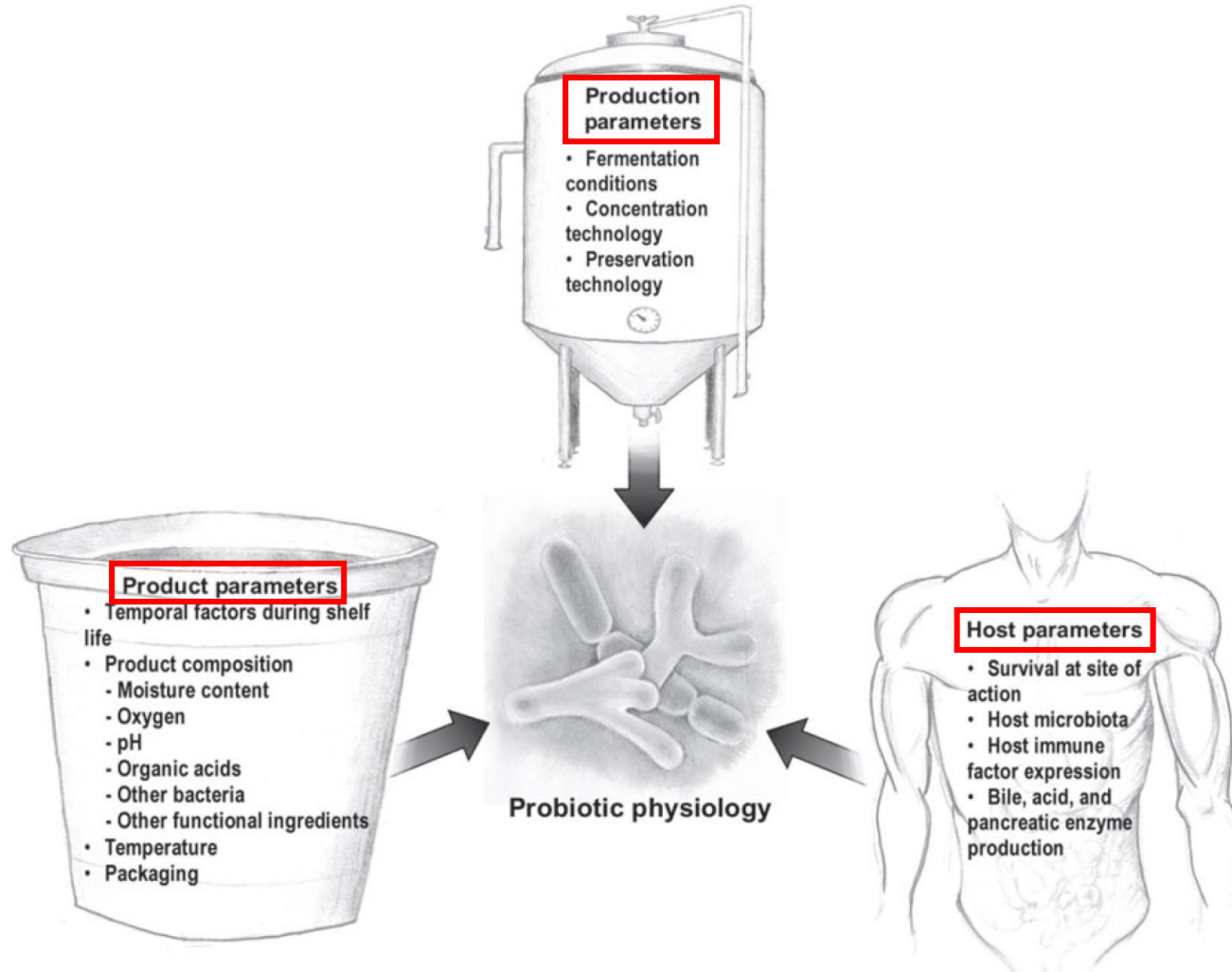
Simulation of a clinical trial



Bifidobacterium spp. DGGE



Conclusions





Conclusions

- ❖ The delivery matrix has an **effect on the viability** of the probiotic strains during shelf-life and passage in the upper GIT
- ❖ The delivery vehicle is likely to **influence probiotic functionality** in many ways including:
 - changes in the physiological status of the probiotic;
 - synergy with other active ingredients (i.e. fibers, bioactives...)
 - fermentation end-products such as organic acids or bacteriocins
 - improving the likelihood of regular consumption through product palatability and incorporation of that product into the diet



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Thank you for your attention



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