Probiotics delivery: does the matrix matter?

Dr Massimo Marzorati

Pre- & Probiotics in Paediatrics
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Introduction

Functional stability

- Hours
- Days
- Months
Introduction

Asset/Liability

Mutualism ↔ Parasitism

Gastrointestinal Resource Management

- metabolism
- cognitive function
- immune development
- regulation cell proliferation
- colonization resistance
- energy salvation
- vitamins

- autism
- allergy
- obesity
- chronic inflammation
- pathogen
- asthma
- cancer
### 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)

<table>
<thead>
<tr>
<th>Fecal microbiota</th>
<th>Breast-fed</th>
<th>Formula-fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ Bifidobacteria</td>
<td></td>
<td>↓ Bifidobacteria</td>
</tr>
<tr>
<td>↑ Lactobacilli</td>
<td></td>
<td>↑ Bacteroides</td>
</tr>
<tr>
<td>↑ Gram⁺ cocci</td>
<td></td>
<td>↑ Coliforms</td>
</tr>
</tbody>
</table>
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
• Probiotics can be defined as live microorganisms which when dosed in adequate amounts confer a health benefit on the host.

Properties of an Ideal Probiotic:
- High adherence to the intestinal wall
- Production of anti-microbial agents for increased intestinal protection
- Naturally part of the human gastrointestinal system
- Must be safe for consumption and effective in providing health benefits
- Must not be pathogenic, possess or pass on antibiotic resistance
- Should be stable against gastric acid, bile, oxygen and enzymes

Govender et al. 2014 - AAPS PharmSciTech
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
SHIME® technology platform

SHIME®: Simulator of the Human Intestinal Microbial Ecosystem
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
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

Marzorati et al. 2015 - LWT - Food Science and Technology
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.](image1)

![Easily opened for sprinkling onto soft food.](image2)

![Contents mixed with soft food for oral administration.](image3)
Use of **microencapsulation (Intelicaps®)** to protect 2 strains of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* subsp. *lactis*

White spherical, uniform particles (size 150 – 600 µm)
Encapsulation improved the viability of the probiotic strains
Delivery of probiotics 2

**Gut metabolism proximal colon**

- **SCFA Probiotic mix**
  - Graph showing concentrations of acetate, propionate, butyrate, and total SCFA over time (T=0h, T=24h, T=48h).

**Effect on the host**

- **NF-kB – AP1 activity**
  - Bar graph showing normalized absorbance percentages.

- **IL10**
  - Bar graph showing normalized concentration percentages.

- **IL8**
  - Bar graph showing normalized concentration percentages.

**Improved SCFA production in the proximal colon and anti-inflammatory activity**
Delivery of probiotics 3

- Cheeses
- Yogurts
- Chocolates
- Milk
- Creams
- Meats

Non-conventional commercial probiotic products

Govender et al. 2014 - AAPS PharmSciTech
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: 
\((7,2 \pm 1,6) \times 10^8\) CFU/mL

Probiotic bacteria in: 
\((6,6 \pm 1,3) \times 10^8\) CFU/mL

62 mg yoghurt N out 
81% yoghurt N removal

324 mg yoghurt N in
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.

Possemiers et al. 2010 - International Journal of Food Microbiology
Simulation of a clinical trial

Control and blank period

Treatment with probiotic chocolate

Delivery of probiotics 4

Bifidobacterium spp. DGGE

Possemiers et al. 2010 - International Journal of Food Microbiology
Conclusions

Sanders and Marco, 2010 - Annu. Rev. Food Sci. Technol
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
Thank you for your attention

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