Role of Food Matrix for Probiotic Effects

W. Kneifel

Department of Food Science and Technology

BOKU – University of Natural Resources and Life Sciences
Vienna

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wolfgang.kneifel@boku.ac.at

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The Spectrum of Probiotic Products

MAIN MESSAGES related to

Differing Formats and Properties of Probiotics

Viability and Stability as Key Issues for Applications – Comparative Evaluation

Tailoring Probiotics - Individual Requirements and Synergies, what Literature Tells

Concluding Remarks and Challenges
Probiotics are well-selected as only those strains...

- ... possessing scientific evidence regarding beneficial effects exerted to the individual
- ... possessing accurate state-of-the-art taxonomic characterisation
- ... possessing evidence to reach the target region at high cell density, viability and stability
- ... fulfilling all safety requirements according to scientific state-of-the-art (GRAS, QPS)

fulfil the quality criteria

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DEFINITION OF PROBIOTICS:

... are live microorganisms which when administered in adequate amounts confer a health benefit to the host......

analytical and technological challenges for product design and development!

„From strain to product“

Probiotic Product Development

To be tested:
- Strain
- Product (strain + matrix)
**Stability ↔ Viability**

**Lifespan of a Microbe**

- **Cell Count**
- **Time**

**Phases**:
- Lag Phase
- Acceleration Phase
- Exponential Phase
- Retardation Phase
- Stationary Phase
- Die-off Phase

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**Techno-analytical approach**

- **LIVE**
  - Culturable, active
  - Viable, but not immediately culturable
  - Viable - but not sufficiently culturable
  - CFUs

- **DEAD**
  - Viable, but non-culturable
  - Dead & non-culturable
**Microbial Cell Density – Viable counts**

<table>
<thead>
<tr>
<th>Lyophilized bacteria</th>
<th>10⁹ - 10¹¹ CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilized, (cryo-)</td>
<td></td>
</tr>
<tr>
<td>protectants</td>
<td></td>
</tr>
<tr>
<td>Encapsulated (resistant against gastric juice)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Active bacteria</th>
<th>10⁶ - 10⁹ CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>in sour protein gel matrix</td>
<td></td>
</tr>
<tr>
<td>Additional protectants</td>
<td></td>
</tr>
</tbody>
</table>

*) viable count
(plate count method, standardized)

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**X-Glu agar plate with colonies of**
*L.acidophilus* (blue) and *L. casei* (white)

**Fluorescent staining of *L. casei***
(Live/Dead status)
Viable Count Losses of Probiotic Products

- Fresh product
- Stored product
- Consumption
- Residual count (Colon)

"Storage Loss"  "Digestion Loss"

...
TALKING ABOUT

**Individual strain characteristics**

- Stability and resistance factors, „Stress-training experience“, cell-wall composition

**Selected culture type**

- Deep-frozen concentrated culture, lyophilized culture, (liquid culture)

**Inherent product composition**

- Protein-fat matrices, sugar concentration, hydrocolloid agents etc.

**Special ingredients**

- Prebiotic carbohydrates (...synbiotics), fruit preparations, other

**Technology**

- Separated fermentation, co-fermentation, post-fermentation supplementation

**Milieu conditions**

- Redox status (oxygen), temperature, pH, acidity, time

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TALKING ABOUT

**Individual strain characteristics**

- Stability and resistance factors, „Stress-training experience“, resuscitation properties, cellular composition

**Inherent product composition**

- Protein-carbohydrate matrices, cryoprotectants, water-binding agents

**Special ingredients**

- Growth activators upon rehydration

**Technology**

- Culture biotechnology, different standards

**Milieu conditions**

- Water activity, redox status (oxygen), temperature, pH, acidity, time

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**Key Criteria of Different Probiotic Formats**

- Bacteria present in different forms of viability, mainly active, but limited (shelf-)life
- Proteinaceous matrix may exert protection to the bacteria in product and in GI tract
- Product acidity supports resorption of nutritive compounds and minerals
- Alternative food carriers (other than dairy) may be envisaged

VS.

- Bacteria present in dormant and dry status, need for resuscitation upon application
- Different techniques may be applied to preserve bacteria in the products on long-term (e.g., [micro]encapsulation)
- Delivery of bacteria strongly depends on degree of re-hydration and re-activation

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**Key Questions Concerning Different Probiotic Product Formats**

- Are probiotic effects of defined strains different, when same strains are applied as dried preparations or through a fermented dairy product?

  ?

- Can probiotic effects of defined strains be enhanced if certain additives or ingredients are added to the product (or preparation)?

  → LITERATURE
Not all lactic bacteria are probiotics, .... but some are

Letter to the Editor


Food matrices have an impact
on the expression of
genes involved in
probiotic-linked mechanisms

Influence of carbohydrates on cell properties of
Lactobacillus rhamnosus


The presence of fructose, mannose,
or rhamnose significantly in the
growth medium significantly reduces
cell surface hydrophobicity,
mucoidity and cell wall composition
of a L. rhamnosus strain.
**A combination of galacto-oligosaccharides and Lactobacillus GG increases bifidobacteria to a greater extent than Lactobacillus GG on its own**

Kekkonen, RA et al., Milchwissenschaft 62 (2007) 326-330

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**Double-blind cross-over study with 44 adults, fruit-milk drink with LGG without and with GOS: fecal bifidobacterial counts more increased in LGG+GOS group**

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**Reconstitution conditions for dried probiotic powders represent a critical step in determining cell viability**


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**Reconstitution and cell integrity study with Bifidobacterium longum and L. johnsonii strains: reconstitution conditions (media, time, protectants etc.) strongly affect viability, activity, morphology and metabolic profile of probiotic strains**

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Challenges in the addition of probiotic cultures to foods


- Too much sugar and dissolved oxygen in the product result in reduced probiotic counts and stability
- Ascorbic acid addition my result in increased *L. acidophilus* counts
- *S. thermophilus* in fermentation culture possesses oxygen-scavenging properties
- *L. delbr. subsp. bulgaricus* in fermentation culture may reduce probiotic count
- Addition of protein hydrolysates and UF retentates may stimulate growth of certain probiotics

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Probiotic dairy products – important prerequisites

**CONSUMER PERCEPTION AND PREFERENCES**

To the consumer, **product and brand** must both be a **credible carrier** of the benefit

There must be a **logic to the whole** that the consumer can accept

**Product must meet sensory preferences**

**Product must fulfil fundamental nutritional requirements**

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Screening: Compositional details of probiotic drinks and yoghurts (Austrian market)

- Carbohydrate range: 4.0 - 14.7 %
- Protein range: 1.3 - 5.2 %
- Fat range: 0.1 – 3.6 %
- Energy range: 121 – 413 kJ
Are consumers sufficiently informed and how is their response to functional foods?

Perceived fit of different combinations of carriers and functional ingredients and its effect on purchase intention.

“...perceived carrier-ingredient fit was related to familiarity with the combination and healthiness of carrier food (status Denmark)”

make probiotics become visible to the consumer

Microencapsulated probiotic bacteria
Conclusions

As long as it is not sufficiently specified for a defined probiotic product and its corresponding target application how many (viable) cells need to be administered, it remains difficult to exactly design a tailored product as many bacteria as possible? more dose-response studies, more studies with final product needed

Degree of matrix-based effects on stability and viability of probiotic strains depends on individual strain characteristics more comparative studies with strains under varying matrix conditions needed to optimize a product

Fermented dairy products as probiotic carriers seem to offer additional beneficial effects to the consumer, if the basic nutrient profile is according to state-of-the art need for re-considering original product formulae to improve the basic quality of the carrier