A Conceptual Framework for Defining Mechanisms of Probiotic Action

Prof. Sarah Lebeer – UAntwerpen (BE)

ILSI Session - How Do Prebiotics and Probiotics Work? – Mechanistic Insights Into Their Function
Probiotics Task Force & Expert Groups

In-depth analyses of probiotic benefits, mechanisms and challenges aiming to advance probiotic knowledge at large

Objectives Expert group mechanisms of action

- to investigate what is currently known about the mechanisms of probiotics action (direct and indirect) in relation to demonstrated (clinical) benefits
- to identify gaps in the current knowledge
- to provide suggestions for the future
### Expert group ‘Probiotic MOA’

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Organization</th>
<th>Country</th>
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<tbody>
<tr>
<td>Prof. Michiel Kleerebezem</td>
<td>Chair</td>
<td>Wageningen University</td>
<td>NL</td>
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<tr>
<td>Dr Johan van Hylckama</td>
<td>Vice Chair</td>
<td>Chr Hansen</td>
<td>DK</td>
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<td>Dr Arthur Ouwehand</td>
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<td>DuPont de Nemours</td>
<td>FI</td>
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<td>Dr Gabriela Bergonzelli &amp; Dr. A. Mercenier</td>
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<td>Nestlé</td>
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<td>Dr Sylvie Binda</td>
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<td>Danone</td>
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<td>Dr Peter Bron</td>
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<td>Dr Gabriele Gross</td>
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<td>Prof. Colin Hill</td>
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<td>University College Cork</td>
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<td>Prof. Reetta Satokari</td>
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Elie Metchnikoff pioneer

• lactic acid bacteria slow aging through their ability to produce lactic acid and inhibition of the protein-fermenting intestinal microbes (1908)
• remarkably similar to definition WHO panel 100y later: “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host”.
• impossible to prove either the clinical efficacy or the underlying molecular mechanism, with the scientific tools available at his time
Where are we now?

• Commercial success, although stagnation in EU
• Probiotic research = mature scientific field > 1000s of papers
• Many clinical studies & meta-analyses document health effects (eg lactose digestion, AAD, NEC, etc.)
• Many mechanistic studies on molecular interactions thanks to molecular tools (mutagenesis, biotechnology, microbiome sequencing, biomarker improvement, etc.)
• HOWEVER, few examples exist where such molecular activities adequately explain the measured or targeted clinical effect
Objective expert group

Molecular mechanism of cross-talk at mucosal level

Cellular responses in the mucosa (local)

Cellular / physiology effect in host (mucosal and systemic)

Clinical benefit (meta-analysis)
Example of lactose digestion

1) Microbe-host crosstalk
   - Bacterial Properties/effectors

2) Physiological functions
   - Host cell response

3) Health benefit
   - Yoghurt/probiotic for lactose intolerance
     - Lactose-provoked Gas-related symptoms
     - Lactose-provoked Diarrhea
     - Improved lactose digestion in the intestine
     - β-galactosidase

«Unchallengable» evidence at all 3 levels

- Solid clinical demonstration of health benefit in lactose intolerant population
- Hydrolysis of lactose prevents increase of osmolarity (→ diarrhea) and load of fast fermentable sugars in the colon (leading to GRS)
- Effect is due to delivery of the microbial β-galactosidase enzyme in the small intestine

EFSA claim:
“Live yoghurt cultures in yoghurt improve digestion of lactose in yoghurt in individuals with lactose maldigestion”

Johan van Hylckama Vlieg, ILSI April 7, 2015
How to rank evidence?

<table>
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<tr>
<th>Health benefit</th>
<th>Physiological function</th>
<th>Microbial interaction</th>
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<td>Reduced lactose intolerance symptoms</td>
<td>Hydrolysis of lactose</td>
<td>Delivery and release of β-galactosidase</td>
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<td>Reduced risk for NEC</td>
<td>Improved competitive exclusion</td>
<td>Direct antagonisms</td>
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<td></td>
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<td>Production of SCFA and other antimicrobial substances</td>
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<tr>
<td></td>
<td></td>
<td>Competition of habitat and/or nutrients</td>
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<td></td>
<td>Improved immune maturation</td>
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**CAUTION:**
- goal is not regulatory but mechanistic explanation
- ‘avoid’ pharma approach
- best clinical evidence does not match most mechanistic explanation

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<td><em>H. pylori</em> eradication</td>
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<td>Production of SCFA and other antimicrobial substances</td>
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<td></td>
<td></td>
<td>Competition of habitat and/or nutrients</td>
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<tr>
<td></td>
<td>Improved mucosal immune response</td>
<td></td>
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<tr>
<td>Shortening of long colonic transit</td>
<td>Stimulate colonic motility</td>
<td>Production of SCFA</td>
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<td>Interaction with 5-HT receptors</td>
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<td>Reduced risk for/shorter RTI</td>
<td>Modulation of mucosal immune system</td>
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<td>Relief of IBS symptoms</td>
<td>Improved pain threshold</td>
<td>Increase in analgesic receptors</td>
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Example of such a meta-analysis

Meta-analysis GIT health

- Independent of species, strain & formulation
- Also yeast & sporeforming bacteria

Core vs strain-specific benefits

**Figure 2** | Possible distribution of mechanisms among probiotics. Some mechanisms might be widespread among commonly studied probiotic genera; others might be frequently observed among most strains of a probiotic species; others may be rare and present in only a few strains of a given species. Evidence is accumulating on a cross-section of probiotic strains that suggest some generalizations can be made beyond strain-specific effects. Abbreviation: SCFA, short-chain fatty acid.

My ‘old’ conceptualised framework

Lebeer et al., 2008, MMBR
Active probiotic molecules?

- Probiotic selection
- Mode of action
- Clinical effects
Example of *L. rhamnosus* GG (LGG)

Strong adhesion to gut wall due to pili (‘zippers’)

Example of *L. rhamnosus* GG (LGG)

Piliation of *Lactobacillus rhamnosus* GG Promotes Adhesion, Phagocytosis, and Cytokine Modulation in Macrophages

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unpublished
Example LGG & immune: not only pili

Lipoteichoic acid (LTA) of LGG interacts with TLR2-6 to induce NF-κB signaling and increase IL-8

Unmethylated CpG-rich DNA motifs of LGG interact with TLR9 on endosomes to stimulate T_h1 responses

Fucose and mannose residues on the pili of LGG interact with DC-SIGN on dendritic cells to increase cytokine expression

Claes et al., 2012 *Microb Cell Fact*

Tytgat et al., 2016 *PLoS One*

Iliev et al., 2005 *Cell Microbiol.*

Iliev et al., 2008 *Scand J Immunol.*
‘One probiotic molecule’ is not key for most probiotic effects

Whether you look from microbial interaction – physiological functions – towards health benefits point of vue
Conceptualised signaling in the gut

1. Microbe-microbe interactions
   - MOA research reductionist
   - Boost by microbiome?

2. Epithelial barrier

3. Immune modulation

Core characteristics probiotics?

- Mostly LABs: lactic acid = inhibitory towards many enteric pathogens & bacteriocin production
- No virulence & toxic properties => pathogens → SAFE
- Adhesion to gut wall is competitive advantage
- Many are Gram-positive
- Important signaling through Toll-like receptor (TLR) 2 linked to barrier function
Many effects via cell wall

(immune) modulators beyond cell wall

- MAMPs: TLR2 activation (LTA, PG, …), TLR9 (DNA)
- Secreted active enzymes (PG hydrolases, proteinases, glycosylhydrolases): potential modulators
  - lactic acid
  - anti-oxidants, vitamins, & other metabolites
Delivery of high dose of biomass

Administration in large amounts (min. $10^8$ CFU/day)
‘New’ framework ILSI expert group

1. Structure and content of microbial biomass
2. Microbiome/ pathogen defence
3. Epithelial barrier function
4. Immune system interaction
5. In situ bioactive production or conversion
6. Neurological system interaction
7. Endocrine function (→ specific receptors)
Responders $\leq$ non-responders
To wrap up: conclusions so far

- Very complex to conceptualise framework for probiotic mechanisms of action
- Biggest challenges
  - BACTERIA: core versus strain-specific effects
  - HOST: responders vs non-responders
- Translational capacity of *in vitro* experiments?

Molecular mechanism of cross-talk at mucosal level
Cellular responses in the mucosa (local)
Cellular / physiology effect in host (mucosal and systemic)
Clinical benefit (meta-analysis)
Perspectives

• What is sufficient evidence to substantiate or clarify a mode of action?
  • Which data do we need for ‘structure – function’ relationships?
  • Taking into account that ‘simple’ biomarkers are not available

• How can research be improved?
  • along the pipeline from selection of strains to clinical trials

We would highly appreciate your input 😊!