Pro- and prebiotics: an evolution that cannot be stopped, despite some questions await answers

Bruno Pot
(Yakult Europe Science Department)

Conflict of Interests

Employee of Yakult Europe

Non-remunerated Board Function at the PRI
What it is all about

• The history and definition of probiotics
• The history and definition of prebiotics
• The consequences on health claim approval in Europe by EFSA
• The complexity of assessing mechanisms of actions on the microbiota
• Questions for the discussion

7000 BC Food fermentations:
- Kefir, Kumis, Beer, Wine...

1857: Louis Pasteur discovered lactic acid bacteria
1878: Joseph Lister: Isolation of lactic acid bacteria from fermented milk (Lactobacillus)
1899: Henry Tissier isolated the first Bifidobacterium
1908: Elie Mechnikov: health aspect: The Prolongation of Life: Optimistic Studies
1935: Minoru Shirota: Yakult (mono culture, commercial probiotic drink)
1953: Werner Kollath: introduces the word Probiotic: active substances that are essential for a healthy development of life
1990: 6 papers on probiotics
2001: FAO/WHO definition
2017: already 1243 publications (x 207 versus 1990)
WHO/FDA definition

- Life microorganisms
- Sufficient quantities
- Health benefit on the host

http://isappscience.org/probiotics/

New categories: the pharma approach, following the third wave of probiotic and therapeutic microbes.

The different generations of probiotics

New challenges

Do we really need to worry about HC approval in the FOOD area?

- Regulatory
- Commercialization
- Marketing
- Scientific support

160 yrs ago

First wave

Second wave

Third wave

Early observations

Hundreds of authors

Large research consortia

Rational selection

Capitalizing on Microbiome insights

Metchnikov
Shiroti

only FOOD
Yoghurt
LAB, bifidus
Yeasts

R&D on surviving the GIT

PHARMA, OTC, (FOOD?)
GRAS / QPS organisms
Gut commensals (cocktails)
FMT-homologs; Phages; …

Physiological effects on the host

Microbe-host cross talk in vitro / in vivo testing

Microbiome Therapies

Preventive and therapeutic Medicine

Ecosystem Engineering

Gut Systems Biology

Marketing

Scientific support
Already now we can see medical terms popping up


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PMCID: PMC4960683

Increased interest ...


Published online 2016 Jul 25. doi: 10.1186/s40413-016-0116-1
PMCID: PMC4960683
And another observation …

Some of this has been attributed to EFSA’s HC approval result

<table>
<thead>
<tr>
<th>Claim type</th>
<th>Authorized</th>
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More than 400 health claim applications on probiotics were NOT approved (> 99.75% failing rate)!

One claim (13.1 Generic claim) succeeded

*Live cultures in yoghurt or fermented milk improve lactose digestion of the product in individuals who have difficulties in digesting lactose*

The condition of use specify that yoghurt or fermented milk should contain at least 10^8 CFU live starter microorganisms (Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus) /gram.

The target population is individuals with lactose maldigestion.

Data based on EU Register of Nutrition and Health Claims, February, 2017
Some of this has been attributed to EFSA’s HC approval result

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Data based on EU Register of Nutrition and Health Claims, February, 2017

http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search

Why probiotic claims were rejected:

- **Insufficient characterization of the FOOD** (not possible to make a scientific assessment of this claimed effect) 53%
  - Should be easy to solve
- **Poor clinical study[ies]** (claimed effect not substantiated) 40%
  - Not so easy to solve (FOOD industry)
    - In healthy people!
    - Requires budget
    - May require MECHANISM of ACTION
- **Poorly defined claim** (claim could not be substantiated) 5%
- **Claim does not cover a beneficial physiological effect** 2%

http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search
Mechanisms important for HC approval

The Lp299v example

ABSTRACT

... The Panel considers that Lp299v is sufficiently characterised. The claimed effect proposed by the applicant is 'increase of non-haem iron absorption'. The target population proposed by the applicant is 'healthy adults who want to increase their iron uptake'. The Panel considers that increasing non-haem iron absorption is a beneficial physiological effect.

In weighing the evidence, the Panel took into account that the results of two double-blind, placebo-controlled, cross-over studies are inconsistent, as one study with some methodological limitations showed a positive effect of Lp299v on non-haem absorption, whereas the other did not show an effect.

The Panel noted that among four single-blind, placebo-controlled, sequential studies at risk of systematic bias, three studies showed a positive effect of Lp299v on non-haem absorption and one did not show an effect.

The Panel also took into account that there is no evidence for a plausible mechanism by which Lp299v could increase non-haem iron absorption in vivo in humans.

The Panel concludes that the scientific evidence is insufficient to establish a cause and effect relationship between the consumption of Lp299v and an increase of non-haem iron absorption.
### Still there is hope...

#### Table 1: Claim Type, Nutrient Substance, Food or Food Category, Claim, Conditions of use of the claim, Restrictions of use, Reasons for non-authorisation, Health

<table>
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<tr>
<th>Claim type</th>
<th>Nutrient substance, food or food category</th>
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<tbody>
<tr>
<td>Art.13(1)</td>
<td>Wheat bran/bran fibre contributes to an acceleration of intestinal transit</td>
<td>The claim may be used only for food which is high in that fibre as referred to in the Annex to Regulation (EC) No 1924/2006. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of at least 10 g of wheat bran fibre.</td>
<td>Increase in faecal bulk</td>
<td>Reduction in intestinal transit time</td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Wheat bran/bran fibre contributes to an increase in faecal bulk</td>
<td>The claim may be used only for food which is high in that fibre as referred to in the Annex to Regulation (EC) No 1924/2006.</td>
<td>Increase in faecal bulk</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Barley grain/barley grain fibre contributes to an increase in faecal bulk</td>
<td>The claim may be used only for food which contains at least 1 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these beta-glucans.</td>
<td>Increase in faecal bulk</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Beta-glucans contribute to the maintenance of normal blood cholesterol levels</td>
<td>The claim may be used only for food which contains at least 1 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these sources per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of beta-glucans from oats, oat bran, barley, barley bran, or from mixtures of these beta-glucans.</td>
<td>Increase in faecal bulk</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Chitosan contributes to the maintenance of normal blood cholesterol levels</td>
<td>The claim may be used only for food which provides a daily intake of 3 g of chitosan. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 3 g of chitosan.</td>
<td>Increase in faecal bulk</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Hydroxypropyl methylcellulose with a meal contributes to a reduction in the post-prandial blood glucose rise after that meal</td>
<td>The claim may be used only for food which contains at least 4 g of HPMC per quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming 4 g of HPMC as part of the meal. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Reduction in post-prandial glycaemic responses</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Arabinoxylan from wheat endosperm as part of a meal contributes to a reduction in the post-prandial blood glucose rise after that meal</td>
<td>The claim may be used only for food which contains at least 8 g of arabinoxylan (AX)-rich fibre produced from wheat endosperm (at least 60 % AX by weight) per 100 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming the arabinoxylan (AX)-rich fibre produced from wheat endosperm (at least 60 % AX by weight) per 100 g of available carbohydrates in a quantified portion as part of the meal. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Reduction in post-prandial glycaemic responses</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Beta-glucans from oats or barley contribute to the reduction of the blood glucose rise after that meal</td>
<td>The claim may be used only for food which contains at least 4 g of beta-glucans from oats or barley per each 30 g of available carbohydrates in a quantified portion as part of the meal. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained by consuming the beta-glucans from oats or barley as part of the meal.</td>
<td>Reduction in post-prandial glycaemic responses</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Consumption of foods containing fructose leads to a lower blood glucose rise compared to foods containing sucrose or glucose</td>
<td>The claim may be used only for food which contains 10 g of pectins per quantified portion. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained by consuming 10 g of pectins as part of the meal. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Reduction in post-prandial glycaemic responses</td>
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<tr>
<td>Art.13(1)</td>
<td>Consumption of pectin with a meal contributes to the reduction of the blood glucose rise after that meal</td>
<td>The claim may be used only for food which provides a daily intake of 100 g of dried plums (prunes). In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 100 g of dried plums (prunes).</td>
<td>Reduction in post-prandial glycaemic responses</td>
<td></td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Dried plums/prunes contribute to normal bowel function</td>
<td>The claim may be used only for food which provides a daily intake of 4 g of glucomannan. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 4 g of glucomannan. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Maintenance of normal bowel function</td>
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Still there is hope...

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<tr>
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<tr>
<td>Art.13(1)</td>
<td>Lactobacillus delbrueckii subsp. bulgaricus</td>
<td>The claim may be used only for food which provides a daily intake of 10 g of lactulose per quantified portion.</td>
<td>The claim may be used only for food which provides a daily intake of 10 g of lactulose per quantified portion. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 10 g of lactulose per quantified portion.</td>
<td>Maintenance of normal blood cholesterol concentrations, reduction in intestinal transit time.</td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Glucomannan (konjac mannan)</td>
<td>The claim may be used only for food which provides a daily intake of 10 g of guar gum.</td>
<td>The claim may be used only for food which provides a daily intake of 10 g of guar gum. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Maintenance of normal blood cholesterol concentrations.</td>
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<tr>
<td>Art.13(1)</td>
<td>Guar gum</td>
<td>The claim may be used only for food which provides a daily intake of 5 g of guar gum.</td>
<td>The claim may be used only for food which provides a daily intake of 5 g of guar gum. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Maintenance of normal blood cholesterol concentrations.</td>
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<tr>
<td>Art.13(1)</td>
<td>Hydroxypropyl methylcellulose (HPMC)</td>
<td>The claim may be used only for food which provides a daily intake of 10 g of HPMC.</td>
<td>The claim may be used only for food which contains 10 g of lactulose in a single quantified portion. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a single serving of 10 g of lactulose per day.</td>
<td>Reduction in body weight, increased in faecal bulk.</td>
</tr>
<tr>
<td>Art.13(1)</td>
<td>Oat grain fibre</td>
<td>The claim may be used only for food which provides a daily intake of 6 g of pectins.</td>
<td>The claim may be used only for food which provides a daily intake of 6 g of pectins. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
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<td>Art.13(1)</td>
<td>Monacolin K from red yeast rice</td>
<td>The claim may be used only for food which provides a daily intake of 10 mg of monacolin K from red yeast rice.</td>
<td>The claim may be used only for food which provides a daily intake of 10 mg of monacolin K from red yeast rice. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Maintenance of normal blood LDL cholesterol concentrations.</td>
</tr>
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<td>Art.13(1)</td>
<td>Oat grain fibre</td>
<td>The claim may be used only for food which provides a daily intake of 6 g of pectins.</td>
<td>The claim may be used only for food which provides a daily intake of 6 g of pectins. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Maintenance of normal blood cholesterol concentrations.</td>
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<td>Art.13(1)</td>
<td>Pectins</td>
<td>The claim may be used only for food which provides a daily intake of 6 g of pectins.</td>
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<td>Maintenance of normal blood cholesterol concentrations.</td>
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<td>Art.13(1)</td>
<td>Resistant starch</td>
<td>The claim may be used only for food in which digestible starch has been replaced by resistant starch so that the final content of resistant starch is at least 14% of total starch.</td>
<td>The claim may be used only for food in which digestible starch has been replaced by resistant starch so that the final content of resistant starch is at least 14% of total starch. Warning of choking to be given for people with swallowing difficulties or when ingesting with inadequate fluid intake - advice on taking with plenty of water to ensure substance reaches stomach.</td>
<td>Reduction in post-prandial glycaemic responses.</td>
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<th>Health relationship</th>
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<td>Art.13(1)</td>
<td>Rye fibre</td>
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<td></td>
<td>Rye fibre contributes to normal bowel function</td>
<td>The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE as listed in the Annex to Regulation (EC) No 1924/2006.</td>
<td>changes in bowel function</td>
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<td>Art.13(1)</td>
<td>Wheat bran fibre</td>
<td>Wheat bran fibre contributes to an acceleration of intestinal transit</td>
<td>The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE as listed in the Annex to Regulation (EC) No 1924/2006. In order to bear the claim information shall be given to the consumer that the claimed effect is obtained with a daily intake of at least 10 g of wheat bran fibre.</td>
<td>reduction in intestinal transit time</td>
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<td>Wheat bran fibre</td>
<td>Wheat bran fibre contributes to an increase in faecal bulk</td>
<td>The claim may be used only for food which is high in that fibre as referred to in the claim HIGH FIBRE as listed in the Annex to Regulation (EC) No 1924/2006. Increase in faecal bulk</td>
<td>Increase in faecal bulk</td>
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One claim only in the PROBIOTIC domain
BUT:
Many more in the PREBIOTIC domain...

http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search

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What are Prebiotics?

- Prebiotics are: selectively fermented, dietary ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health.

- A prebiotic targets the microbiota already present within the ecosystem, acting as a selective ‘food’ for the target microbes with beneficial consequences for host.
5 000 000 yrs ago fructans available, but consumed? digestion-inhibiting compounds and plant toxins present in many below-ground food sources probably limited their role as staples in early diet until technological adaptations, such as fire, were introduced (Ragir, 2000).

9 000 yrs ago direct and indirect evidence extensive exploitation of prebiotic rich plants such as agave, camas and wild onion

1978: Yazawa et al. some carbohydrates, especially fructo-oligosaccharides (FOS), are selectively fermented by bifidobacteria and have the capacity to stimulate their growth in mammals

1995: Gibson and Roberfroid, define prebiotics and describe their health benefit through human trials with FOS and inulin ‘a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of limited number of bacteria in the colon, and thus improves host health’

1997: Boubnik et al., 10 g/day transgalacto-oligosaccharides reduce breath hydrogen

2000: Gibson et al. human trial to assess the prebiotic activity of soybean oligosaccharides

2002: Tuohy et al. reported the prebiotic effect of lactulose in a controlled human study.

But not less controversial than pro-!

2004: Gibson et al. review updating the concept of prebiotics: other components can be suitable for inclusion in the diet and may exert specific effects upon gut microbiota:

- A prebiotic has to
  1. resist gastric acidity, hydrolysis by (mammalian) enzymes and GI absorption,
  2. be fermented by the intestinal microbiota,
  3. stimulate selectively the growth and/or activity of intestinal bacteria associated with health and well-being.

So the definition can include: germinated barley foodstuffs, oligodextrans, gluconic acid, gentio- and pectic-oligosaccharides, mannan oligosaccharides, lactose, glutamine and hemicellulose rich substrates, resistant starch and its derivatives, oligosaccharides from melibiose, lactoferrin-derived peptides and N-acetylchito-oligosaccharides.

2006: EU ban on antimicrobial growth promoters in agri-business production

2007: Roberfroid: only inulin and TOS fulfil the criteria for prebiotic classification (as proof is available)
- Certain homology between ‘probiotics’ and ‘lactic acid bacteria’ in a broader meaning
- Need of clinical studies and MECHANISM of action to make the difference?

2015: Bindels et al., broader definition (selectivity and specificity are less critical)

2016: Prebiotics: why definitions matter: The discussion continuous!

Why definitions matter...

- A consensus among scientists on the most appropriate definition of a prebiotic is necessary to enable continued use of the term:
  - by scientists
  - by regulators
  - by the food industry
  - by consumers and health care professionals
- Recent developments in community-wide sequencing and glycomics have revealed that more complex interactions occur between putative prebiotic substrates and the gut microbiota than previously considered.
- SO: The complexity is huge and the study methods limited…
- BUT understanding the mode of action may help to describe and understand.
As proven by the positive EFSA news

ABSTRACT

... Non-digestible carbohydrates including FOS are resistant to hydrolysis and absorption in the small intestine and do not contribute to post-prandial glycaemia. This opinion applies to non-digestible carbohydrates (e.g. non-starch polysaccharides, resistant oligosaccharides and resistant starch) which should replace sugars in foods or beverages in order to obtain the claimed effect.

= simple rationale …

The Panel considers that the food constituent, non-digestible carbohydrates, which is the subject of the health claim, and the food constituent (i.e. sugars) that non-digestible carbohydrates should replace in foods or beverages, are both sufficiently characterised in relation to the claimed effect. The Panel considers that a reduction of post-prandial glycaemic responses might be a beneficial physiological effect.

= biologically / clinically relevant in healthy people... (risk of diabetes development) = easy to measure (biomarker / mechanism is available)

Coming presentations: potential or established mechanisms of probiotic action

Source: O’Toole and Cooney (2008)

But how much do we know on the molecular mechanisms? But also: if we talk about foods, do we need to know detailed mechanisms?

(1) competition for dietary ingredients as growth substrates
(2) bioconversion of, for example, sugars into fermentation products with inhibitory properties
(3) production of growth substrates, for example, EPS or vitamins, for other bacteria
(4) direct antagonism by bacteriocins
(5) competitive exclusion for binding sites
(6) improved barrier function
(7) reduction of inflammation, thus altering intestinal properties for colonisation and persistence
(8) stimulation of innate immune response (by unknown mechanisms).
Conclusion

• Some provocative questions for the next speakers and for the discussion…
  • Are we indeed moving from FOOD to PHARMA
  • Is EFSA playing a role in this?
  • Do we need more fundamental research ‘rather than’ or ‘on top of’ new clinical trials?
  • Are definitions indeed that important?
  • Are mechanisms indeed that important?

Thanks to www.ilsie.eu for the invitation

Thanks to you for your attention!