Probiotics and Prebiotics as Functional Ingredients

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Outline

• Definition of Probiotic, Prebiotic and Synbiotic
• Health Beneficial of Probiotic and Prebiotic
• Application in Food:
  – Dairy Product
  – Non-Dairy Product
Probiotic, Prebiotic and Synbiotic

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic = for life (Greek)</td>
<td>Live microorganisms administered in adequate amounts which confer a health benefit to the host FAO/WHO (2001)</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health</td>
</tr>
<tr>
<td>Synbiotic</td>
<td>A mixture of probiotic and prebiotic</td>
</tr>
</tbody>
</table>

ILSI: a food can be considered functional if it can demonstrate satisfactorily that it has beneficial effect on one, or several specific functions in the organism, beyond the normal nutrition effects that improve the state of health and well being, or reduce the risk of a disease.

Probiotics and Prebiotics Market

- The global probiotic market at $24.2 billion in 2011.
  - Predicted 6.8% growth from 2012 to 2017.
  - Probiotic dairy products was the highest market share accounting for almost 80% in 2011.
  - Expected to reach $23.9 billion by 2017.
- The biggest markets are Europe and Asia-Pacific.
- Prebiotics market is projected to reach nearly $1.2 billion and $225 million, respectively, by the year 2015, in the European and the U.S. market.

Probiotic ingredients
Worth $797.6 million in 2008 and increase to $917 million by the end of 2013, 2.8 percent growth.
(http://www.naturalproductsinsider.com/)
Why do we need probiotics-prebiotics?

Factors influencing gut microflora
- Prematurity (newborn)
- Type of feeding (infant)
- High-stress life style
- Eating habit/Dietary intake
- Age of the host
- Antibiotic treatment
- Interaction between microbial groups

Health promoting effect of Probiotics

| Benefit       | • Activate local macrophages to increase antigen presentation to B lymphocytes and increase secretory immunoglobulin A (IgA) production both locally and systemically
|               | • Modulate cytokine profiles
|               | • Induce hyporesponsiveness to food antigens
|               | • Digest food and compete for nutrients with pathogens
|               | • Alter local pH to create an unfavorable local environment for pathogens
|               | • Produce bacteriocins to inhibit pathogens
|               | • Scavenge superoxide radicals
|               | • Stimulate epithelial mucin production
|               | • Enhance intestinal barrier function
|               | • Compete for adhesion with pathogens
|               | • Modify pathogen-derived toxins
|               | • Hypocholesterolemic action
|               | • Anticancer activity

Probiotic effect on infant:

- Shorten duration of diarrhea
- Prevention of diarrhea
- Improvement of atopic eczema incident and severity
- Reducing incidence of neonatal necrotizing enterocolitis
- Reduction in infection of the preterm infant

Desirable Properties of Probiotics

1. Preferably human origin
2. Ability to resist upper GI tract secretions (acid, bile, enzymes)
3. Adherence to human intestinal cells
4. Colonization of the human intestinal tract
5. Production of antimicrobial substances
6. Antagonism against carcinogenic/pathogenic organisms
7. Safety in food and clinical use
8. Clinically-proven health benefits
9. Technologic properties for commercial viability
Guidance for assessment probiotics (WHO, 2001)

- Identification of the genus and species of probiotic by combination of phenotypic and genotype → identification up to strain level
- In vitro testing to delineate the mechanism of probiotic effect
- Substantiation of the clinical health benefit of probiotic against human trial
- Safety assessment

Safety assessment

- Pattern of antimicrobial drug resistant
- Metabolic activities
- Side effects noted in humans during clinical trials and after marketing
- Toxin production and hemolytic potential
- Lack of infecting in animal trials
### Probiotic microorganism

<table>
<thead>
<tr>
<th>Microflora</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacilli</td>
<td>Lactobacillus acidophilus</td>
</tr>
<tr>
<td></td>
<td>L. rhamnosus</td>
</tr>
<tr>
<td></td>
<td>L. reuteri</td>
</tr>
<tr>
<td></td>
<td>L. casei</td>
</tr>
<tr>
<td></td>
<td>L. gasseri</td>
</tr>
<tr>
<td></td>
<td>L. plantarum</td>
</tr>
<tr>
<td></td>
<td>L. johnsonii</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Bifidobacterium bifidum</td>
</tr>
<tr>
<td></td>
<td>B. longum</td>
</tr>
<tr>
<td></td>
<td>B. breve</td>
</tr>
<tr>
<td></td>
<td>B. infantis</td>
</tr>
<tr>
<td></td>
<td>B. adolescentis</td>
</tr>
<tr>
<td>Enterococci</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td></td>
<td>E. faecium</td>
</tr>
<tr>
<td>Lactococci</td>
<td>Lactococcus lactis subsp lactis</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td></td>
<td>Bacillus clausii</td>
</tr>
<tr>
<td>Yeast</td>
<td>Saccharomyces cerevisiae (boulardii)</td>
</tr>
</tbody>
</table>

Not all species/strains are equal
Not all yoghurts are equal
Probiotic properties are strain dependent

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### Example of probiotics marketed in the world

- **Lactobacillus casei** Shirotà Yakult, Japan
- **Lactobacillus crispatus** CTV05 Gynelogix, USA
- **Lactobacillus reuteri** MM53 BioGaia, Sweden
- **Lactobacillus casei** F19 Arla Foods, Denmark/Sweden
- **Bifidobacterium lactis** HNO19 Danisco, France
- **Lactobacillus rhamnosus** GG Valio, Finland
- **Lactobacillus acidophilus** NCFM Rhodia, USA
- **Lactobacillus acidophilus** NCFB 1748
- **Lactobacillus johnsonii** LA1 (NCC 533) Nestle’, Switzerland
- **Lactobacillus fermentum** RC-14 Urex, Canada
- **Lactobacillus casei** DN-114 001 Danone, France
- **Bifidobacterium animalis** DN-173 010
- **Lactobacillus plantarum** 299v Probi AB, Sweden
- **Lactobacillus rhamnosus** 271
- **Lactobacillus casei** CRL 431 Chr. Hansen, USA
- **Bifidobacterium lactis** BB-12
- **Lactobacillus acidophilus** LA-5
Example of oral administration trials of a specific probiotic strain

World Gastroenterology Organisation, 2008

<table>
<thead>
<tr>
<th>Disorder, action</th>
<th>Probiotics</th>
<th>Recommended dose in the trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment of acute infectious diarrhea in children</td>
<td>L. rhamnosus GG, L. reuteri ATCC 55730, L. acidophilus + B. infantis (Infloran strains)</td>
<td>10^{10}–10^{11} cfu, twice daily 10^{10}–10^{11} cfu, twice daily 10^{8} cfu each, three times daily</td>
</tr>
<tr>
<td>Prevention of C. difficile diarrhea in adults</td>
<td>L. casei DN-114 001, L. acidophilus + B. bifidum (Cultech strains)</td>
<td>10^{10} cfu, twice daily 2 × 10^{10} cfu each, once daily</td>
</tr>
<tr>
<td>Adjuvant therapy for H. pylori eradication</td>
<td>L. rhamnosus GG</td>
<td>6 × 10^{9} cfu, twice daily</td>
</tr>
<tr>
<td>Prevention of necrotizing enterocolitis in preterm infants</td>
<td>B. infantis, S. thermophilus, and B. bifidum, L. acidophilus + B. infantis (Infloran strains)</td>
<td>0.35 × 10^{8} cfu each strain, once daily 10^{9} cfu each, twice daily</td>
</tr>
<tr>
<td>Alleviates some symptoms of irritable bowel syndrome</td>
<td>B. infantis 35624, L. rhamnosus GG</td>
<td>10^{8} cfu, once daily 6 × 10^{9} cfu, twice daily</td>
</tr>
</tbody>
</table>

Prebiotics:
Criteria required for a prebiotic effect

- Resistance of the prebiotic to degradation by stomach acid, mammalian enzymes or hydrolysis
- Fermentation (breakdown, metabolism) of the prebiotic by intestinal microbes
- Selective stimulation of the growth and/or activity of beneficial microorganism in the gut
- Induce luminal or systemic effects that are advantageous to the host
- Stability in processing treatment

Teitelbaum & Walker 2005, Roberfroid 2007
Prebiotic mechanisms

Outlook for Functional Food Ingredients
Bogor, 6 February 2014

Śliżewska et al., 2012

Effect of prebiotics

• Increasing the numbers of bifidobacteria in the colon

• Increasing calcium absorption
• Increasing fecal weight
• Shortening gastrointestinal transit time
• Possibly, lowering blood lipid levels
Prebiotics

Fructans
- Composed of fructose polymers which are generally linked to the moiety of a terminal glucose
- Resistant to enzymes in the digestive tract of human and pass through the upper portion of the human GI tract, reach the colon and are fermented by colonic microflora producing SCFA
  - Inulin:
    - Saccharides of the form $\alpha 1-2 [\beta-\text{Fru} 1-2]n$ with $n>10$
    - Inulin is a naturally occurring storage carbohydrate commonly found in leeks, onions, wheat, asparagus, garlic, Jerusalem artichoke and chicory
  - Fructo-oligosaccharides (FOS)
    - composed of a mixture of oligosaccharides consisting of glucose linked to fructose units by $\beta-(1,2)$ links with DP: 2-5.
    - The commercial production of FOS is enzymatic process
- Added to various foods: biscuits, drinks, yoghurt, breakfast cereals, table spreads

Prebiotics

Galacto-oligosaccharide (GOS)
- Galacto-oligosaccharides containing oligosaccharides of the form Glu $\alpha1-4 [\beta-\text{Gal} - 1-6]n$ with $n=2-5$, produced commercially from lactose by $\beta$-galactosidase
- Resemble GOS naturally found in human milk
- Used in many food applications:
  - infant formulas, dairy products, sauces, soups, breakfast cereals, snack bars, ice creams, beverages, bakery products, animal feeds, and as sugar replacements to increase texture and mouthfeel of foods, and act as bulking agents
  - The mixture of 90% GOS and 10% inulin (at concentration of 0.8 g/dL) are used in infant formulas (EU Directive December 2006)
Prebiotics

• Lactulose
  – a synthetic disaccharide which was used originally as a laxative.
  – has also been shown to increase *Lactobacilli* and *Bifidobacteria* and significantly decrease bacteroides in mixed continuous fecal culture.
  – In some clinical trials lactulose has been demonstrated to have potential for use as a prebiotic.

Emerging prebiotics

Data on probiotic effect mostly from in vitro studies or animal studies but do not yet have human data to support them

• Xylo-oligosaccharides
  – Chains of xylose molecules linked by β1–4 bonds which are produced enzymatically by hydrolysis of xylan from birch wood, oats, or corn cobs.

• Resistant starch
  – The fraction of starch that escapes digestion in the upper GI tract and that reaches the colon to be fermented by the colonic microbiota

• Pectic oligosaccharides
  – A complex galacturonic acid-rich polysaccharide which occurs naturally in the cell walls of higher plants and acts as a cement-like material for the cellulosic components of the plant cell wall

• Soybean oligosaccharides
  – Main oligosaccharides are raffinose and stachyose
  – Human trial shows some prebiotic activity
### Prebiotic effect of various oligosaccharides

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Nondigestibility</th>
<th>Fermentation</th>
<th>Selectivity</th>
<th>Prebiotic status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin and oligofructose</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Galactooligosaccharides</td>
<td>Probable</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Probable</td>
<td>?</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Isomaltoligosaccharides</td>
<td>Partly</td>
<td>Yes</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Lactosucrose</td>
<td>NA</td>
<td>NA</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Xylooligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Soybean oligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Glucooligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
</tbody>
</table>

Roberfroid, 2007

### Use of Probiotics and Prebiotics in food

#### Prebiotics
- Safety of ingredient is a must and good sensory properties desirable
- Good prebiotics are stable under heat and when dried, can be stored at room T for months
- A daily dose of 5-8g/d FOS or GOS has a prebiotic effect in adults
- Doses higher than 20 g/day might induce some side effects, such as increased flatulence or abdominal bloating.

#### Probiotics
- Probiotic foods must show, at least, the same performance in any sensory test as conventional foods.
- Should contain sufficient amount of live bacteria to provide health benefit as proven by clinical/human trials.
Probiotic and Synbiotic Products

Single strain or Multiple strains Probiotics

Foods and drink
Fermented Non-fermented
Dairy products Non-dairy products

Dietary Supplement
Capsules Chewable tablets Sachet/ Powder

Supplemented with prebiotics and/or other active components
Synbiotic products

Application of Probiotic in Dairy Industry

- Having a good buffering capacity, milk is a good carrier for probiotic strains.
- Fermented products:
  - Yoghurt and fermented milk drink
  - Cheeses
- Non-fermented products:
  - Ice cream and milk based dessert
  - Powdered milk for infant
  - Butter, Mayonnaise, Fat spread

Annual per-capita consumption of fermented milk products (including yogurt):
- Europe: 35–45 L/person/year, particularly in Scandinavian countries and The Netherlands,
- North America: 4–5 L/person/year
Probiotic in yoghurt

- The probiotic microorganisms may be added together with the starter culture.
- The probiotic microorganisms may be grown in one batch of milk to achieve a high viable count, including:
  - another batch of milk is fermented with traditional starter culture.
  - The two batches are then mixed together.
- Probiotic microorganism(s) may be used as starter culture, the fermentation may be longer.
- Addition of microencapsulated probiotic.

Effect of prebiotic on probiotic yoghurt

- Addition of lactulose potentially enhance viability of bifidobacteria.
- Inulin addition to co-cultures and cocktail:
  - Enhanced products firmness,
  - Increase in microbial growth induced by metabolic interactions among lactic acid bacteria and partial inulin metabolism.

(Oliviera et al., 2011)
### Probiotic in cheeses

- Introduce as adjunct cultures together with lactic starter cultures
  - Two stage fermentation:
    - Fermentation with probiotic bacteria for 2 h followed by fermentation with starter culture
    - The lactic starter culture grow faster than probiotic bacteria
- Addition of microencapsulated probiotic
- Addition of dried probiotic cultures during salting of curd on semi-hard and hard cheese
- Addition of freeze dried culture to matrix of Cheddar cheese following cheddaring and salting
- Addition of fermented cream dressing in cottage cheese
  - Cream dressing is added for flavour and texture development

### Probiotics in ice cream and frozen milk based dessert

- The ice cream matrix is a good vehicle for probiotic culture
- During manufacturing ice cream, agitation and freezing stress detrimental to probiotic bacteria
- Application:
  - Blending the ice cream mix and probiotic cells prior to freezing
  - Fermented milk is combined with ice cream mix → frozen yoghurt ice cream
- Improvement stability of probiotic:
  - Addition of prebiotic (inulin and oligofructose):
  - use of microencapsulated probiotics
Probiotic in infant formula

• Probiotic in infant formula to help introduction of bifidobacteria and lactobacilli into GI of bottle fed infant:
  • Formula feeding results in more diverse group of microorganisms:
    • Bifidobacteria, facultative anaerobs, bacteriodes and clostridia
  • Breast milk feeding: less complex
    • Tend to harbour greater number of bifidobacteria
• Use dried preparation of probiotic bacteria for infant or follow up formula
• Legislation for infant formulas is extremely strict, and strains used in such products must be well documented.

Probiotic in Non Dairy Products

Why non dairy products?

• Demand for non dairy probiotics:
  – Vegetarian
  – Lactose intolerance
  – Low consumption of milk

Development of probiotic products:
  Soy based
  Fruit and vegetable based
  Cereal based
Non-dairy probiotic in the market

<table>
<thead>
<tr>
<th>Product</th>
<th>Base</th>
<th>Probiotic/Microorganism</th>
</tr>
</thead>
</table>
| Grainfields    | Grains, beans and seeds (oats, maize, rice, alfalfa seed, pearl barley, linseed, mung beans, rye grain, wheat, millet) | Lb. Acidophilus  
Lb. Delbrueckii, S. bouardi  
and S. cerevisiae                                         |
| Vita Biosa     | a mixture of aromatic herbs and other plants                         | combination of lactic acid and yeast cultures               |
| Proviva        | oatmeal gruel                                                        | Lb. plantarum 299v                                         |
| Gefillus       | Fruit juice                                                          | Lb. rhamnosus GG                                            |
| Bioprofit      | Fruit juice                                                          | Lb. rhamnosus GG  
and Propionibacterium freudenreichii ssp. shermanii JS   |
| Biola          | Fruit juice                                                          | Lb. rhamnosus GG                                            |

Some non-dairy products that has been developed

<table>
<thead>
<tr>
<th>Fruit and vegetable based</th>
<th>Cereal based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable-based drinks</td>
<td>Cereal-based puddings</td>
</tr>
<tr>
<td>Fermented banana pulp</td>
<td>Rice-based yogurt</td>
</tr>
<tr>
<td>Fermented banana</td>
<td>Oat-based drink</td>
</tr>
<tr>
<td>Beets-based drink</td>
<td>Oat-based products</td>
</tr>
<tr>
<td>Tomato-based drink</td>
<td>Yosa (oat-bran pudding)</td>
</tr>
<tr>
<td>Many dried fruits</td>
<td>Maize-based beverage</td>
</tr>
<tr>
<td>Green coconut water</td>
<td>Wheat, rye, millet, maize, and other cereals</td>
</tr>
<tr>
<td>Peanut milk</td>
<td>fermented</td>
</tr>
<tr>
<td>Cranberry, pineapple, and orange juices</td>
<td>probiotic beverages</td>
</tr>
<tr>
<td>Ginger juice</td>
<td>Malt-based drink</td>
</tr>
<tr>
<td>Grape and passion fruit juices</td>
<td>Soy based</td>
</tr>
<tr>
<td>Cabbage juice</td>
<td>Nonfermented soy-based frozen desserts</td>
</tr>
<tr>
<td>Carrot juice</td>
<td>Fermented soymilk drink</td>
</tr>
<tr>
<td>Noni juice</td>
<td>Soy-based stirred yogurt-like drinks</td>
</tr>
<tr>
<td>Onion</td>
<td>Other nondairy foods</td>
</tr>
<tr>
<td>Probiotic banana puree</td>
<td>Starch-saccharified probiotic drink</td>
</tr>
<tr>
<td>Nonfermented fruit juice beverages</td>
<td>Probiotic cassava-flour product</td>
</tr>
<tr>
<td>Blackcurrant juice</td>
<td>Meat products</td>
</tr>
</tbody>
</table>

Summarised from Prado et al. (2008)
Incorporation of probiotic in non-dairy products

- Fruit juice:
  - Probiotic included as ingredient, they do not multiply
  - Need protection from the acidic condition of fruit juice → use microencapsulated
- Storage at room temperature for cereal product, confectionary create overwhelming challenge for probiotic stability
- Fermented vegetables can offer a suitable media to deliver probiotics. *Probiotic of L. rhamnosus, L. casei and L. plantarum* are better adapted to the vegetable during fermentation
- Remarkable of flavour and aromatic impact may influence consumer acceptance

Technological aspects of probiotics

- The preparation should remain viable in large production
- It should be stable during the storage and use
- The preparation should be able to survive in the intestinal ecosystem
- The host must get benefits to lodge probiotics

- Microencapsulation Technique
**Microencapsulation Technique to improved viability**

- Microencapsulation: a process whereby the cells are retained within the encapsulating membrane

- Entrapment in gelatin, calcium alginate, xanthan-gellan or vegetable gums

- Encapsulated cells can be dried to produce cell powder/graunule (freeze drying, spray drying, fluidized bed drying)

**Perspectives**

- Product development on probiotics, prebiotics and synbiotic products are promising with their application beyond traditional fermented milk, while dairy products remain leading.

- Research to substantiate preventive, therapeutic health benefits, mechanism of action, optimal intake, selection of specific strains for a targeted outcome and mode of delivery is needed

- Viability, physiological and metabolic activity of probiotic bacteria in a food product at the point of sale are important consideration for their efficacy

- Acceptance by consumer on the non dairy products product is fundamental to get consumer appreciation
Thank you

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