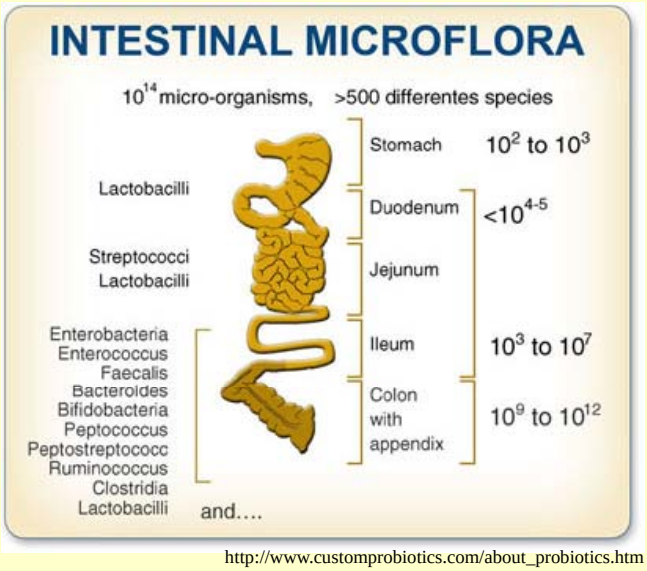
Trend and Innovation of Pro and Prebiotics in Dairy Industry

Lilis Nuraida
Southeast Asia Food and Agricultural Science & Technology (SEAFAST) Center
and
Department of Food Science and Technology
Bogor Agricultural University

Outline

- Microbiota of Human Gut
- Probiotic and Heatlh
- Application of probiotic in dairy products
- Improvement of viability of probiotic in the products
- Prebiotics
- Synbiotic

Microbiota of Human Gut



http://www.customprobiotics.com/about_probiotics.htm

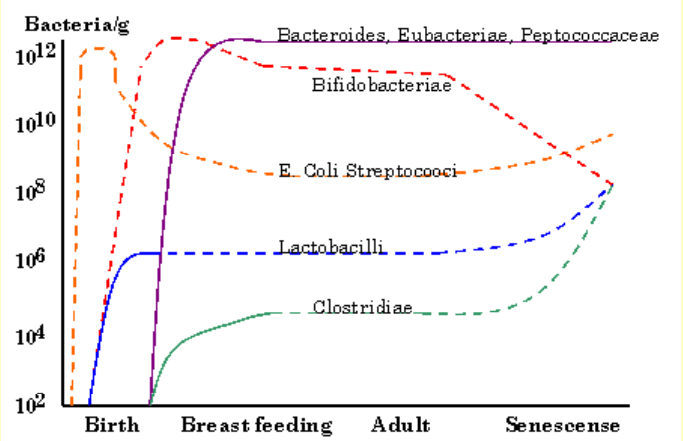
Lilis Nuraida - June 2011

Influences on composition of the gastrointestinal microbiota

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

Lilis Nuraida - June 2011

Changes in the fecal Flora with increased age



Mitsuoka, 1990

Bifidobacteria accounts up to 95% of the saccharolytic bacteria in large intestine of breastfed newborn and 25% in the adult colon

Lilis Nuraida - June 2011

How Can We Restore the Microbiota Balance ??

Probiotics

Live Beneficial Bacteria

Prebiotics

Foods for Beneficial Bacteria

Synbiotics

Combination of probiotics with prebiotics
Improves survivability and implanting of probiotics
More effective than probiotics alone

Lilis Nuraida - June 2011

Probiotics

Probiotic = for life (Greek)

Live microorganisms administered in adequate amounts which confer a beneficial health benefits to the host

FAO/WHO (2001)

Lilis Nuraida - June 2011

Probiotic market

- Around 30% of the global population buys into the probiotic dairy sector on a regular basis
  - representing a major part of the US$85bn global functional foods market
  - The US, Western Europe and Japan account for over 70%.
- In 2008 the global probiotics market: over US$15.7bn (over 18% of the global functional foods market).
- Since 2003, the global probiotics market has more than doubled in value terms, and is currently rising by almost 15% per annum.
- Approximately 70 probiotic-containing products marketed in the world.

http://www.report linkers.com/

Lilis Nuraida - June 2011

Desirable Properties of Probiotics

1. Ability to resist upper GI tract secretions (acid, bile, enzymes)
2. Adherence to human intestinal cells
3. Colonization of the human intestinal tract
4. Production of antimicrobial substances
5. Antagonism against carcinogenic/pathogenic organisms
6. Safety in food and clinical use
7. Clinically-proven health benefits
8. Preferably be isolated from the same species as the intended use
9. Technological properties for commercial viability

SEAFAST CENTER

Lilis Nuraida - June 2011

Probiotic and Health

1. Nutritional improvement of foods
2. Suppression of lactose intolera
3. Inhibition of enteric pathogen
4. Metabolize and detoxify harmful s
5. Hypocholesterolemic action
6. Anticancer activity
7. Stimulation of the immune system

Strain dependent

Atributed to certain lactobacilli and bifidobacteria used as probiotics

SEAFAST CENTER

Lilis Nuraida - June 2011

Lactic Acid Bacteria that have been identified as probiotic microflora

Microflora	Species
Lactobacilli	*Lactobacillus acidophilus* *L. rhamnosus* *L. reuteri* *L. casei* *L. gasseri* *L. plantarum* *L. jhonsonii*
Bifidobacteria	*Bifidobacterium bifidum* *B. longum* *B. Breve* *B. infantis* *B. adolescentis*
Enterococci	*Enterococcus faecalis* *E. faecium*
Lactococci	*Lactococcus lactis subsep lactis*

Lilis Nuraida - June 2011

Example of successfull probiotic strains in the market

Strain	Origin
L. casei Shirota	Human
L. rhamnosus GG	Human
L. johnsonii LA1	Human
L. acidophilus	Not known

Others: L. reuteri, L. paracasei, Bifidobacterium bifidum, B. longum, B. brevis, B. infantis, B. animalis (B. animalis Bb12)

Lilis Nuraida - June 2011

Application of Probiotic in Dairy Industry

- Significant increase in consumer awareness of probiotic cultures
- Probiotic cultures is one of the top consumer trends in foods delivering health benefits.
- Dairy products are the main vehicle for incorporation probiotic bacteria

- Yoghurt and fermented milk drink
- Cheeses
- Ice cream and milk based dessert
- Powdered milk for infant
- Others: Butter, Mayonnaise, Fat spread

Yoghurt and fermented milk drink

- Convetional yoghurt is fermented by *L. bulgaricus subsp delbruekii* and *S. Thermophilus*
  - not very resistant to the bile released into the small intestine →not maintained alive in the gastrointestinal tract in very high numbers
- Type of product:
  - Non-drinkable fermented milks (spoonable)
  - Drinkable fermented milk (including cultured buttermilk, yoghurt drink and dairy drink)

Incorporation of probiotic into fermented milk

- Add the probiotic microorganisms together with the starter culture
  - Probiotics do not usually grow markedly during mixed fermentation
- The probiotic microorgnisms may be grown in one batch of milk to achieve a high viable count, 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

Several aspect must be considered during the production of probiotic fermented milk drink

- Many probiotic strain grow slowly in non-supplemented milk due to limited proteolytic activity
  - Supplemented with hydrolised protein, whey derivatives, or amino acid
- The production condition are often unsuitable for their growth
  - Optimum temperature for probiotic isolated from human is 37^{o}C
- The metabolites of probiotics may be undesirable due to formation off flavor
  - Bifidobacteria produce acetic acid and lactic acid in the proportion 3:2 which give vinegar like taste

Probiotic Cheeses

- Natural cheese has proven to be a good carrier for these cultures.
- Studies have suggested that consuming probiotics in a cheese matrix is favorable for the viability of probiotics through the digestive tract.

Led to the marketing of several varieties of cheese containing added probiotic cultures

- Cheese with probiotic microorganisms: Feta type cheese, Chedar, Edam, Emmental, Cheese-based dip , etc.

Probiotics in Cheeses

- Probiotic microorganisms must survive the cheesemaking process and entire shelf-life of the cheese
- Must not produce metabolites that are detrimental to the quality of cheese
- Should not interfere with the normal activity of other essential microorganisms in the cheese
  - Should not produce antimicrobial compounds
- Should be able to grow on starter culture media

Introduction of probiotic microorganism into cheeses

- Introduce as adjunct cultures together with lactic starter cultures
  - Risk of losing large numbe rof cells to whey or domination of lactic starter cultures
- Addition of dried probiotic cultures during salting of curd on semi-hard and hard cheese
- Addition of fermented cream dressing in cotage cheese
  - Cream dressing is added for flavour and texture development

Ice cream and frozen milk based dessert

- Freeze stress must be considered with respect to viability during manufacture and extended storage
- Addition:
  - Direct, i.e. blend the ice cream mix and probiotic cells prior to freezing
  - Involve fermentation of the milk for proliferation of probiotic bacteria prior to blending with ice cream mix
- Protection of the probiotic cells againts freeze damage is important (use cryoprotectant)
- Impact of probiotic bacteria on flavor should be considered as ice cream is not fermented product

Probiotic in infant formula

- At birth, the gastrointestinal tract is sterile, but it rapidly becomes colonized by microbes associated with the birthing process and the delivery environment

- Two stages in the formation of human microflora:
  - Acquisition of microorganisms by transmission of the mothers' microorganisms
    - Normal birth vs Caesarian
  - Succesive colonization of the different habitat:
    - Breast fed vs bottle fed
    - Early use of antibiotics and sterile environments such as incubators.

Breast Fed vs Bottle Fed

Breast milk or formula feeding in the neonatal period have different effects on the colonization of the gastrointestinal tract :

- Formula feeding results in more diverse group of microoorganisms:
  - Bifidobacteria, facultative anaerobs, bacteriodes and clostridia
- Breast milk feeding: less complex
  - Tend to harbour greater number of bifidobacteria

Probiotic in infant formula to help introduction of bifidobacteria and lactobacilli into GI of bottle fed infant
Use dried preparation of probiotic bacteria for infant or follow up formula

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

Not all probiotics act similarly

Factors should be considered in respect to viability of probiotic in dried products

- Drying method
- Type and size packaging
- Temperature and humidity of storage
- Powder quality
- Rehydration procedure
- Handling of rehydrated product

Viablity of probiotic bacteria

- Viability, physiological and metabolic activity of probiotic bacteria in a food product at the point of sale are important consideration for their efficacy
- They have to survive during shelf life of a food, transit through high acidic and alkaline conditions in the gastro-intestinal tract

- Probiotic bacteria should be present in a food to minimum concentration of 10^6 cfu/g or the daily intake should be about 10^9 cfu/g
- Fermented Milk and Lactic Acida Bacteria Beverages Association, Japan: minimum 10^7 cfu/ml to be present in dairy products

SEAFAST CENTER

Lilis Nuraida - June 2011

Viablity of probiotic bacteria

- Factors affecting viability:
  - Strain
  - Interaction between species present
  - Production of hydrogen peroxide
  - Availability of nutrient, growth promoters and inhibitors
  - Concentration of sugar
  - Dissolve oxygen level and oxygen permeation through package
  - Innocualtion level
  - Fermentation time

SEAFAST CENTER

Lilis Nuraida - June 2011

Improvement of viability probiotic bacteria in dairy products 1

- Selection of bacterial strain(s)
  - Acid and bile tolerance is strain specific
- Type of packaging container
  - Bifidobacteria is anaerobic, while lactobacilli is microaerophilic
- Rate of innoculation
  - Some probiotic bacteria grow poorly in milk →use a large innoculum size
- Two-stage fermentation
  - Acid and hydrogen peroxyde produce by yoghurt starter culture may be detrimental to probiotic culture → adding probiotic bacteria after fermentation or adding yoghurt starter culture at later stage

Improvement of viability probiotic bacteria in dairy products 2

- Microencapsulation technique
  - Freezing causes freeze-injury
  - Microencapsulation: a process whereby the cells are retained within the encapsulating membrane
  - Entrapment in gelatin, calcium alginat, xanthan-gellan or vegetable gums
  - Encapsulated cells can be dried to produce cell powder/granule (freeze drying, spray drying, fluidized bed drying)
- Supplementation of milk with nutrient
- Use of oxygen scavengers, i.e. ascorbic acid
- Addition of cystein

Prebiotics

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

Oligosaccharides

Gibson and Roberfroid, 1995

Lilis Nuraida - June 2011

Criteria required for a prebiotic effect

- Resistance of the prebiotic to degradation by stomach acid, mammalian enzymes or hydrolysis:
  - Neither be hydrolized or absorbed in the upper part of the gastrointestinal tract
- Fermentation (breakdown, metabolism) of the prebiotic by intestinal microbes
- Selective stimulation of the growth and/or activity of beneficial microorganism in the gut
  - Alter the colonic microenvironment toward healthier composition
- Induce luminal or systemic effects that are advantageous to the host

Teitelbaum & Walker 2005, Roberfroid 2007

Lilis Nuraida - June 2011

Prebiotic effect of various oligosaccahrides

Carbohydrate	Nondigestibility	Fermentation	Selectivity	Prebiotic status
Inulin and oligofructose	Yes	Yes	Yes	Yes
Galactooligosaccharides	Probable	?	Yes	Yes
Lactulose	Probable	?	Yes	Yes
Isomaltoligosaccharides	Partly	Yes	Promising	No
Lactosucrose	NA	NA	Promising	No
Xylooligosaccharaides	NA	NA	Promising	No
Soybean oligosaccharides	NA	NA	NA	No
Glucooligosaccharides	NA	NA	NA	No

Roberfroid, 2007

Human milk oligosaccharides

- Oligosaccharides are one of the main components of human milk, which contains, on average, 10 g/L of neutral oligosaccharides and 1 g/L of acidic oligosaccharides (Boehm & Stahl, 2003)
- The composition of human milk oligosaccharides is very complex and more than 100 different oligosaccharide-like structures are known.
- Human milk oligosaccharide are resistance to enzymatic digestion → Provide substrate for bifidobacteria

Prebiotic in infant formula

- Prebiotic added to infant formula primarily to stimulate the growth of bifidobacteria and lactobacilli → pattern the microflora of breast-fed infant

Mixture of GOS (90%) and FOS (10%) alter stool microbiology, pH, and consistency of formula-fed infant
Decrease incident of constipation

Prebiotic in infant formula

The addition of a mixture of 10% inulin and 90% GOS in a concentration of 0.8 g/dL to infant formula was recognized safe by the European Commission in December 2001. This was confirmed in the last EU directives of December 2006 [Commission directive 2006/141/EC on infant formulae and follow-up formulae (33)] with the following wording in Annex 1: "fructo-oligosaccharides and galacto-oligosaccharides may be added to infant formula… their content shall not exceed 0.8g/% in a combination of 90% oligogalactosyl-lactose and 10% high molecular weight fructosyl-saccharose. … other combinations may be used." The document

Use of Prebiotics in food

- 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.

Synbiotic: Probiotics and Prebiotics – Combined Benefits

- **Conceptually, the desirable state or optimising intestinal flora can be achieved by either probiotics or prebiotics.**
- **Prebiotics help probiotics become established.**
- **Probiotics provide the most physiological beneficial strains.**
- **Prebiotics help maintain high populations of probiotics.**
- **Combination of prebiotics and probiotics will provide synergistic benefits** **(Gibson, 1998)**

Example synbiotic products in the market:
Yoghurt: Probiotic cultures + Inulin
Powdered formula for infant: Probiotic cultures + GOS/FOS

Thank You

SEAFAST Center

http://seafast.ipb.ac.id/

lilis@seafast.org