Paper No.: 07

Paper Title: TECHNOLOGY OF MILK AND MILK PRODUCTS

Module – 18: Probiotic and Synbiotic Dairy Products

INTRODUCTION

Inappropriate nutrition is recognized as a primary factor in unachieved genetic potential, reduced mental and physical performance, and increased susceptibility to disease. In searching for effective dietary intervention strategies, a new class of food, functional food, has evolved rapidly and many new terms such as probiotic, prebiotic, nutraceutical and FOSHU (Food for Specified Health Use) have emerged in response. The term "functional food" originated in Japan in the 1980s, when it was used by the industry to describe foods fortified with specific ingredients imparting certain health benefits. There are many possible definitions for the term functional food; however, functional food is usually described as food that promotes health beyond the provision of basic nutrition.

Probiotic bacteria are live microbial strains that, when applied in adequate doses, beneficially affect the host animal by improving its intestinal microbial balance. Probiotic foods are food products that contain a living probiotic ingredient in an adequate matrix and in sufficient concentration, so that after their ingestion, the postulated effect is obtained, and is beyond that of usual nutrient suppliers. The most common probiotic dairy products worldwide are various types of yoghurt, other fermented dairy products (e.g. cultured buttermilks in Finland), various lactic acid bacteria drinks ('Yakult-type') and mixtures of probiotic (fermented) milks and fruit juice. Probiotic cheeses, both fresh and ripened, have also been launched recently.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Prebiotic foods are food products that contain a prebiotic ingredient in an adequate matrix and in sufficient concentration, so that after their ingestion, the postulated effect is obtained, and is beyond that of usual nutrient suppliers. Synbiotics are mixtures of pro- and prebiotics that beneficially affect the host by improving the survival and implantation of selected live microbial strains in the gastrointestinal tract.

PROBIOTICS

The first records of ingestion of live bacteria by humans are over 2,000 years old. However, at the beginning of this century probiotics were first put onto a scientific basis by the work of Metchnikoff at the Pasteur Institute in Paris. Metchnikoff hypothesized that the normal gut microflora could exert adverse effects on the host and that consumption of 'soured milks' reversed this effect. Metchnikoff refined the treatment by using pure cultures of what is now called *Lactobacillus delbrueckii* subsp. *bulgaricus* which, with *Streptococcus salivarius* subsp. *thermophilus*, is used to ferment milk in the production of traditional yoghurt.

A formal probiotic definition is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Over the years, many species of micro-organisms have been used as probiotics. They consist not only of lactic acid bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria) but also *Bacillus* spp. and fungi such as *Saccharomyces* spp. and *Aspergillus* spp.

STRAINS USED IN MANUFACTURE OF PROBIOTIC DAIRY PRODUCTS

The probiotic strains used in dairy products most commonly belong to *Lactobacillus* and *Bifidobacterium* genera (Table 1).The characteristics of probiotic strains vary, and hence each strain requires individual study. The primary requirement of a probiotic strain is that it should be adequately identified with methods based on genetics, and that the strain should be defined in the text of the product package. This makes it possible to analyze the scientific data behind any claims made. Some probiotic strains are sufficiently proteolytic to grow excellently in milk, but others need growth stimulants. Sometimes the texture or the taste of a milk product fermented with a probiotic does not meet with consumer demand hence probiotic strains are used as adjunct with standard starter cultures. Probiotics can be added before the fermentation of the milk, or part of the milk can be fermented separately with the probiotic strain and then mixed after the fermentations.

Table 1 The most common species of bacteria used in probiotic dairy products

- *Lactobacillus acidophilus* group

  L. acidophilus, L. johnsonii,

  L. gasseri, L. crispatus

- *L. casei/paracasei*

- *Bifidobacterium lactis*
- *B. bifidum*
- *B. infantis*
- *B. breve*

- *L. rhamnosus*
- *L. reuteri*
- *L. plantarum*
- *B. animalis*
- *B. adolescentis*

HEALTH BENEFITS OF PROBIOTIC FOODS

Main positive effects associated with probiotics include cholesterol and/or triglyceride reduction, anti-tumor properties, protection against gastroenteritis, improved lactose tolerance and stimulation of the immune system through nonpathogenic means. For centuries folklore has suggested that fermented dairy products containing probiotic cultures are healthful. Recent controlled scientific investigation has supported some of these traditional views, suggesting the value of probiotics as part of a healthy diet. In addition, the emergence of some new public health risks suggests an important role for effective probiotics.

Probiotics may be the avenue to provide a safe, cost-effective, "natural" approach that adds a barrier against microbial infection. Some of the reported benefits of probiotic organisms are summarized in Table 2.

SELECTION CRITERIA FOR PROBIOTICS

In selecting a probiotic microorganism for use in foods, the strain must be identified at the genus, species and strain levels. Understanding the complex interactions among microorganisms in the intestinal ecosystem requires methods of differentiating a strain of interest from other strains of the same species contained in the indigenous microbiota. According to the FAO/WHO guidelines for probiotic use, specific health benefits observed in research employing a specific strain may not be extrapolated to other, closely-related, strains

In humans, the most commonly used vector involves fermented milk products and ‘over the counter’ freeze-dried preparations of lactic acid bacteria in capsules. Recently, the market has expanded to include other foods such as flavoured drinks and pharmaceutical preparations such as tablets. Selection criteria for efficacious probiotics are as follows:

- Must exert a beneficial effect on the consumer
- Non-pathogenic and non-toxic
- Contain a large number of viable cells
- Survive in the gastrointestinal tract

- Good sensory properties
- Preferably be isolated from the same species as the intended use

The theoretical basis for selection of probiotic micro-organisms illustrated in Table 3, which includes:

- Safety
- functional behaviour (survival, adherence, colonisation, anti-microbial production, immune stimulation, anti-genotoxic activity and prevention of pathogens such as *Helicobacter pylori, Salmonella*, *Listeria* and *Clostridium*)
- technological aspects (growth in milk, sensory properties, stability, phage resistance, viability in processes).

Table-2. Selected Health-Related Attributes of Probiotics Used in Fermented Milks

Type of Effects	**Published Health Benefits**
Physiological effects	• Bile, pH resistant strains, enzymatic activity • Production of bacteriocins • Antagonistic effect against pathogens
Actions on the digestive tract	• Enhancement of lactose digestion in lactase-deficient people • Prevention of intestinal disturbances • Adherence to human intestinal cell line cultures (in vitro) • Stimulation of intestinal immunity in animal models • Stabilization of Crohn's disease • Regulation of intestinal motility
Alterations of the intestinal microflora	• Balance of intestinal bacteria • Increase in fecal bifidobacteria • Decrease of fecal enzyme activities thought to play a role in conversion of pro-carcinogens to carcinogens • Decrease of fecal mutagenicity • Colonization of intestinal tract
Actions on diarrhea	• Prevention and/or treatment of acute diarrhea • Prevention and/or treatment of rotavirus diarrhea • Prevention of antibiotic-associated diarrhea • Treatment of relapsing Clostridium difficilediarrhea • Treatment of persistent diarrhea
Systemic	• Immune enhancer

effects	• Stimulation of phagocytic activity • Stimulation of γ interferon production by human blood mononuclear cells in culture • Reduction of hypertension in animal models and in humans • Antagonism against carcinogenic bacteria • Beneficial effects in superficial bladder and colon cancer • Reduction in the risk of various cancers • Alleviation of clinical symptoms in children with atopic dermatitis • Reduction of serum cholesterol

In general, strains for pilot testing should be selected based on established *in vitro* scientific data. Safety criteria and functional properties for successful probiotics should include the following specifications:

- Strains for human use are preferably of human origin.
- They are isolated from healthy human GI tract.
- They have a history of being non-pathogenic even in immunocompromised hosts.
- They have no history of association with diseases such as infective endocarditis or GI disorders.

Table 3: Desirable properties of probiotic bacteria

Desirable properties	**Desired effect**
Human origin	Ability to maintain verified viability, speciesspecific effects on health
Acid and bile stability	Maintenance of viability in the intestine
Adherence to human intestinal cells	Maintenance of mild acidity in the intestine, antagonism against pathogens, competitive exclusion
Colonisation of the human gut	Maintenance of colonising properties, antagonism against pathogens, competitive exclusion

Production of anti-microbial substances	Antagonism against pathogens, competitive exclusion
Antagonism against pathogenic bacteria	Antagonism against pathogens, competitive exclusion (in intestinal tract and oral cavity)
Safety in human use	Tested safety in animal models and human use, accurate strain identification (genus, species)

Processing issues in developing probiotic foods

Functional foods with probiotics are now well established in the world market. This is a result of intensive research and development within the industry and the academic field. Probiotic foods should retain specific probiotic strains at a suitable level during the storage time experienced by them. Before probiotic strains can be delivered to consumers, they must first be able to be manufactured under industrial conditions, and then survive and retain their functionality during storage as frozen or freeze-dried cultures, and also in the food products into which they are finally formulated. Additionally, they must be able to be incorporated into foods without producing off-flavours or deteriorated textures. Today, research efforts are being made in incorporating probiotic encapsulation technology into foods to ensure the viability and stability of probiotic cultures. All the commercial probiotic strains used in the manufacture of probiotic foods must demonstrate the following criteria:

- The probiotic strain should be able to produce concentrated cultures of each specific strain in levels above 10^{10} CFU/g with good storage properties at low temperature
- The probiotic strain of interest must be able to produce probiotic foods with help of a supporter culture
- The probiotic strain should be able to ferment milk together with supporter cultures without inhibition of the growth of any of the added strains
- Strain should be able to produce probiotic foods with levels of the specified probiotic strain within $10^{6} – 10^{8}$ CFU/g OF product
- The probiotic strain should remain stable at low temperature of storage for three weeks

- The probiotic strain should not impart any off flavour during storage period.
- The probiotic strain must produce probiotic foods with acceptable stability and viscosity

THE CONCEPTS OF PREBIOTICS AND SYNBIOTICS

A prebiotic is defined as 'a 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'. It is a substance which modifies the composition of the colonic microflora in such a way that a few of the potentially health-promoting bacteria (especially lactobacilli and bifidobacteria) become predominant in numbers. A synbiotic is the product in which both a probiotic and a prebiotic are combined in a single product. It is defined as 'a mixture of a probiotic and a prebiotic that beneficially affects the host by improving the survival and the implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria'. The prebiotics developed so far are the non-digestible oligosaccharides and especially the non-digestible fructans among which chicory fructans play a major role.

PREBIOTICS

Prebiotics have been defined as 'non-digestible food ingredients that, when consumed in sufficient amounts, selectively stimulate the growth and/or activity of one or a limited number of microbes in the colon resulting in documented health benefits'. With probiotics, there is an emphasis on viability and the dose consumed. Prebiotics, on the other hand, positively influence selected microbes. Since both types of ingredients have the intestine as functional target, it is obvious that the two can be mixed and may reinforce each other's effect. Thereby creating a synbiotic: 'a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or activating the metabolism of one or a limited number of health promoting bacteria, and thus improving host welfare'.

The clue to prebiotic compounds is that they are not digested in the upper gastrointestinal tract, because of the inability of the digestive enzymes to hydrolyze the bond between the monosaccharide units. They act as soluble fibers and are digested in the colon, enhancing microbial activity and stimulating the growth mainly of bifidobacteria and lactobacilli.

Such compounds, which are either partially degraded or not degraded by the host and are preferentially utilized by bifidobacteria as a carbon and energy source, are referred to as "bifidogenic factors." Some bifidogenic factors of commercial significance include fructo-oligosaccharides, lactose derivatives such as lactulose, lactitol, galacto-oligosaccharides, and soybean oligosaccharides.

Consumption of higher doses of prebiotics may encourage the formation of gas, flatulence and intestinal discomfort. The end-products in the gut fermentation are mainly short chain fatty acids (acetic, propionic and butyric acid), lactic acid, hydrogen, methane and carbon dioxide. Short chain fatty acids, especially butyric acid, are known to act as an energy source for enterocytes.

The main dairy products enriched with prebiotics are yoghurts and yoghurt drinks, but spreads, fresh cheeses and milks are also on the market. Galacto-oligosaccharide, a milk-based prebiotic, is derived from lactose by the β-galactosidase enzyme. It is a natural prebiotic of human breast milk, and facilitates the growth of bifidobacteria and lactobacilli in breast-fed infants. Galacto-oligosaccharides are commercially used principally in Japan and other parts of Asia.

In Europe inulin and fructooligosaccharides are widely used in various functional foods, including dairy-based products. Inulin is a group of fructose polymers linked by β (2–1) bonds that limit their digestion by enzymes in the upper intestine. Their chain lengths range from 2 to 60. Oligofructose is any fructose oligosaccharide containing two to ten monosaccharide units linked with glycosidic linkage. Both inulin and fructooligosaccharides (oligofructoses) are extracted from plant material (e.g. chicory) or synthesised from sucrose. The role of inulin and the oligofructoses in a food matrix is bi-functional. They do not increase the viscosity of a milk product but give a richer texture to liquid products and spreads.

SYNBIOTICS

When combining both a probiotic and a prebiotic in a single food product, the expected benefits are, as stated in the synbiotic definition, an improved survival during the passage of the probiotic bacteria through the upper intestinal tract and a more efficient implantation in the colonic microbiota together with a stimulating effect of the prebiotic on the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (e.g. bifidobacteria).

Synbiotics are much more than just a mixture of pre- and probiotics. As the name suggests, a synergy must exist between the two components and hence not just any mixture will be a synbiotic. The majority of yogurts marketed in Australia, the United States, and Europe in recent years contain probiotic bacteria and some form of prebiotics. Such combination products are referred to as "synbiotics." Japan is the world leader in probiotic and prebiotic products. A synbiotic is a marriage of the concepts of probiotics and prebiotics.

The advantages are that a commercial probiotic with known benefits can be used and the prebiotic aids the establishment of the organism in the complex colonic environment. The survival of the probiotic part of a synbiotic can be determined *in vitro* using a model of the human gut.

Whereas the concept of prebiotic has become very popular since its introduction in 1995, the concept of synbiotic has, up to now, not really been applied to the development of new foods especially functional foods. Furthermore, even when new products indeed contain a mixture of pro- and prebiotics they are seldom presented or defined as synbiotics. This concept thus remains open for validation and further research is needed.

REFERENCES

Danone Research Centers (1998) Mechanisms of Protection of the Digestive Tract, Paris, France: John Libbey. *Eurotext*. 1998.

De Vrese, M. and Schrezenmeir, J. (2001) 'Pro and prebiotics', *Innov Food Technol*, May/June, 49–55.

Gibson, G. R. and Roberfroid, M. B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* 125, 1401–1412.

Gibson, G.R. and Williams, C.M. (2000) Functional foods Concept to product, Woodhead Publishing Ltd, CRC Press LLC, USA.

Mattila-Sandholm, T. and Saarela, M. (2000) Probiotic functional foods, In: Functional foods

Concept to product, Glenn R. Gibson and Christine M. Williams (Eds.), Woodhead Publishing Limited, Cambridge, UK.

Mattila-Sandholm, T. and Saarela, M. (2003) Functional dairy products, Vol. 1, Woodhead Publishing Ltd, CRC Press LLC, USA.

Mattila-Sandholm, T. and Salminen, S. (1998) Up-to-date on probiotics in Europe, *Gastroenterol Int*, 11 (1), 8–16.

O'sullivan, M. G. (1996) 'Metabolism of bifidogenic factors – an overview', *Bull Int Dairy Fed*, 313, 23–30.

Roberfroid, M.B. (1998) Prebiotics and synbiotics: concepts and nutritional properties, *British Journal of Nutrition*, 80, Suppl. 2, S197–S202.

Saarela, M. (2007) Functional dairy products, Vol. 2, Woodhead Publishing Ltd, CRC Press LLC, USA.

Sabikhi, L. (2003) Developments in Probiotic Foods, 16th CAS Course Compendium on "Application of Biotechnology in Dairy and Food Processing" (4th Nov. to 24th Nov., 2003), NDRI, Karnal-132 001, India.

Salminen, S. and Tanaka, R. (1995) Annual review on cultured milks and probiotics, *IDF Nutrition Newsletter*, 10:47-50.

Wollowski, I., Rechkemmer, G. and Pool-Zobel, B. L. (2001) 'Protective role of probiotics and prebiotics in colon cancer', *Am J Clin Nutr*, 73(2 Suppl), 451S–455S.