I T E M S NEWS

PROBIOTIC FOOD

MICRO-ORGANISM FOR PROBIOTIC FERMENTED MILKS

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What is a probiotic milk

According to recent studies, probiotic food is defined as "a live microbial food ingredient that is beneficial to health". A more specific and detailed definition could be milk derivate fermented food with good nutritional properties and containing a high level of alive probiotic micro organisms. It is a food very similar to the regular yogurt, not too acidic, beneficial to people's health, with the ability to maintain a good intestinal equilibrium.

Essential micro-organisms to prepare probiotic fermented milk

Micro-organisms used to prepare probiotic fermented milk belong mainly to two different groups:

a) milk bacteria group
b) *bifidus* bacteria group

Since many micro-organism species are involved, we can further classify milk bacteria in relation to a specific species:

a1. *Lactobacillus acidophilus* including:

- *Lactobacillus gasseri*

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- *Lactobacillus crispatus*
- *Lactobacillus amylovorus*
- *Lactobacillus gallinarum*
- *Lactobacillus johnsonii*

a2. *Lactobacillus casei* including:
- *Lactobacillus paracasei*
- *Lactobacillus rhamnosus*

a3. Others (being independent one to each other):
- *Lactobacillus reuteri*
- *Lactobacillus delbrueckii subsp. Bulgaricus*
- *Lactobacillus salivarius*
- *Lactobacillus plantarum*
- *Streptococcus thermophilus*

Bifidus bacteria can be classified in:
- *Bifidobacterium longum*
- *Bifidobacterium infantis*
- *Bifidobacterium bifidum*
- *Bifidobacterium animalis*
- *Bifidobacterium breve*
- *Bifidobacterium lactis*

Characterustics of probiotic micro-organisms

Micro-organisms able to produce fermented probiotic milk should have specific, technological and functional characteristics.

1. Important general characteristics:
- To originate from an intestinal environment
- To be biologically safe and with no side effects even for immune-depressed people
- To be resistant to a low pH (gastric and pancreatic secretions)

2. Technological characteristics:
- To be able to survive during the conservation of a fermented milk at an acid pH
- Do not initiate secondary metabolic reactions during conservation that could lead to proteolysis and subsequent increased acidity and bad flavours

3. Functional characteristics:
- To be able to adhere to the intestinal epithelium
- To be able to reduce the pro-carcinogenic activity of some intestinal enzymes
- To be able to stimulate the immune system
- To be able to grow inside the bowel
- To be able to inhibit intestinal pathogens

Tassonomic classification of probiotic micro-organism families

Since many new fermented types of milk have been commercialized, the specific tassonomic definition and characteristics of each probiotic food involved is very important. They could be classified according to phenotypic, genotypic or probiotic characteristics. Many bacteria stains from the *Lactobacillus* family have been classified and employed for the preparation of probiotic fermented milks (Table 1).

Table 1. Lactobilla employed in commercialized probiotic fermented milks

SPECIES	STAIN	PRODUCERS
L. Rhamnosus	GG	VALIO DAIRY, FINLAND
L. Rhamnosus	271	PROBI, SWEDEN
L. Plantarum	229V	PROBI, SWEDEN
L. Casei	CRL431	CHR, HANSEN, USA
L. casei	DN 114.001	DANONE, FRANCE
L. Johnsonii	LA1 (LJ1)	NESTLÈ, SWITZERLAND
L. Acidophilus	NCFM	RHODIA, USA
L. Reuteri	SD2112	BIO GAIA, USA
L. Casei	SHIROTA	YAKULT, JAPAN
L. Acidophilus	STB-2062	SNOW BRAND MILK PRODUCTS, JAPAN

PROBIOTIC ACTIVITIES AND INTERACTIONS WITH THE IMMUNE SYSTEM

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Despite an increased consumption of probiotic fermented milks, few scientific studies have been published in this field, and the specific role of probiotic bacteria has not yet been established. Publications untitled "Facts or fantasy?" is a symptom of the scepticism in this area, probably due to the huge number of utilized micro-organisms and their different characteristics. Furthermore, regular yogurts, made without intestinal bacteria, were used together in these studies with the probiotic ones. The recent commercialization of fermented milk with specific and well characterized bacteria stains gives the opportunity to improve the knowledge of probiotic activities for the costumers' health. Studies published so far are mostly related to interactions between probiotic bacteria stains and other intestinal bacteria or to the direct effects on humans.

Interactions with other intestinal bacteria

As proposed by Metchnikoff in the past, milk bacteria ingestion could reduce the number of putrefying bacteria in the bowel. This concept evolved towards the intuition that human health is due to the ability in preventing intestinal colonization rather than the fight between different bacteria stains. In other words, adding safe bacteria to the food makes it possible to improve the immune defence capability. In order to do this, probiotic bacteria should be able to bypass the resistance against colonization: this is an unspecific mechanism used to favour bacteria already present in the bowel and to prevent new colonization.

For this reason, it is very important to determine the effective dose to allow the ingested bacteria to colonize. Controlled studies have shown that a dose of 10 milliards bacteria per day should be given to human volunteers in order to increase resistance to gastric environment and to obtain colonization in 100% of the studied subjects. This number is indicative since each bacteria stain has a specific recommended dose (Table 2).

The prevention and cure of gastrointestinal disease has been scientifically proven using the recommended doses. Some studies performed on newborns fed with fermented milk with

Table 2. Recommended daily dose for each probiotic lactobillus

STAIN	EFFECTIVE DOSE IN UFC/DAY	BIBLIOGRAPHY
L. casei Shirota	6,5 x 109	Shimizu - Shibamoto, 1965
L rhamnosus GG	109 x 1010	Saxelin et al, 1991
L. Plantarum 299 V	5 x 108	Johansson et al, 1993
L. acidophilus NCFB 1748	3 x 1011	Lidbeck et al, 1993
L. reuteri	1x 108-1011	Wolf et al, 1995
L. rhamnosus DSM 6594	16 x 109	Ahrne' et al, 1995
L. johnsonii LA 1	1 x 1010	Schiffrin et al, 1995

L.casei and *L. Acidophilus* showed a decreased incidence of diarrhoea and a faster weight gain. The same authors, using the same milk, had shown the same statistically significant results in babies hospitalized for *E. Coli, Salmonella* and *Shigella infections*. Since similar results were obtained in babies with rotavirus infection, the WHO Committee suggests the use of fermented virus in case of infantile diarrhoea.
Animal studies had shown that *lactobacilli* can lead to an immune system enhancement and an improvement in mucosal intestinal function. Prevention of repetitive infections by *Clostridium difficile* has been shown using a specific *lattobacillus* at high doses.

Interactions with immune system

Intestinal microflora is a huge source of antigens for the intestinal mucosa. Recent studies have shown the importance of probiotic bacteria as immune stimulators. At the beginning, some animal studies showed a correlation between probiotic batteria and immune system response mediated by macrophages. These studies showed a better response to *L.casei* and *L. acidophilus* compared to *S. thermophilus*, with variability due to the specific used stain and dose. Recent studies have also shown that the use of fermented milk with *lactobacilli* and *bifidobacilli* leads to an increased phagocyte activity against *E .coli* in human volunteers. Interesting results have been obtained studying the link between fermented milk and GALT (Gut Associated Lymphoid Tissue), the immune system related to intestinal mucosa. GALT interacts with the systemic immune system: it is made of few specific organs such as Peyer's pacis, M cells and many other cells involved in the immune system (B lymphocytes, T lymphocytes and macrophages). *In vitro* stimulation of GALT has been shown in animal and human studies only for few stains and they reported a different GALT stimulation using different bacilla stains. Studies performed in humans with fermented milks containing some specific *Lactobacillus* and *Bifidobacterium* showed a positive immune system response against *Salmonella typhi* infection and in patients affected by the Crohns disease or chronic youth arthritis. What is still yet unknown is the comprehension of the molecular mechanisms acting on immune system.

NUTRITIONAL ASPECTS OF FERMENTED MILK

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Introduction

Fermented milks have a higher nutritional composition compared to regular milks, as a consequence of the presence of added powder milk and the bacteric fermentation. Furthermore fermented milks have a large variety of flavour and density resulting from lactic acid production and the presence of specific aromatic components. Acetaldehyde, dyacetyl and acetone give particular flavour to yogurt. Nutritional profiles of fermented milks results also improved because of a higher content of proteins, carbohydrates, lipids, minerals and vitamins. Nutritional composition of different types of yogurts (the commonest kind of fermented milk eaten in the west countries) is shown in table 3 and 4.
The nutritional value is the capability of food to ensure nutritional needs satisfaction, to protect

Table 3. Nutritional composition of different yogurts for 125 g (from Syndifrais, 1997)

	Half skimmed milk yogurt	Skimmed milk yogurt	Whole milk yogurt	Skimmed milk yogurt with fruit	Half skimmed milk yogurt with fruit	Whole milk yogurt with fruit	Flavour Half skimmed milk yogurt with sugar
Proteins (g)	5.4	5.6	5.2	4.5-5	4.6	4	4.8
Lipids (g)	1.5	0.3	4.3	0.3	1.3	3.3	1.3
Carbohydrates (g)	6.2	6.5	6.2	13.7-22.5	21.2	23.7	17.5
Calcium (mg)	185	185	194	175	175	175	175
Kcal	60	51	84	75-106	115	140	101
KJoules	251	213	351	313-443	481	585	422

against some diseases and to have a diet effect. Recently few yogurts containing *lattobacillus* or *bifidobacillus* have been commercialized. These probiotic bacteria represent a living micro flora able to improve the intestinal equilibrium (Fuller, 1989). Furthermore, they can stick to the intestinal wall building a barrier against other organisms and stimulating the immune system.

Macronutrients

As a consequence of the photolytic actions of probiotic micro-organisms, peptides and free amino acids are present in higher concentrations in fermented milk compared to regular milk. Some authors suggested that lactic fermentation can improve digestion of aminoacid. If this reaction doesn't lead to real advantages for healthy people, benefits are evident in people with bowel diseases showing an improvement in the gastric kinetic and aminoacid adsorption.

Fermentation also leads to modification of milk fatty acids. Free linoleic acid appears to be transformed into the linked form after a biohydrogenation process with subsequent delivery of new free fatty acids such as stearic and oleic acid depending on the specific bacterial utilized stain.

It is well known that the main result of the fermentation process is the transformation of 20-30% of lactose in galactose, glucose and lactic acid that decreases to 0.8-1%. Positive effects of lactic acids are: a) a capability in preserving the product; b) a delicious flavour; c) a good biodisponibility of calcium and other minerals; d) the inhibition of intestinal bacterial growth. L+ and D- lactic acids are present in the yogurt in different proportion according to different condition of preparation and conservation. D- form is metabolized slower and its urinary secretion is higher compared to the L+ from. Human adults are supposed to use both of them at the same time. After the break down of lactose, galactose concentration rapidly increases reaching a 1-1.5%. Galactose can be absorbed very easily by the gut and metabolized in glucose within the tissue. The most frequent cause of lactose intolerance is due to a lactases deficit at the intestinal mucosa level. No-hydrolyzed lactose undergoes fermentation at the bowel level because of the presence of intestinal flora producing organic acids and gas (Carbonic hydrogen, methane, hydrogen). Hydrogen will be then eliminated throughout the lungs. In conclusion, bad lactose absorption could be demonstrated by simply measuring an increased hydrogen concentration in the exhaled air after lactose digestion. Children usually develop lactose intolerance as a conse-

quence of gut pathology, although this is a primarily disease in adult subjects. In fact the lactase activity is higher in childhood and it starts decreasing after the introduction of solid food until a residual 10% activity in adulthood. Recent studies have shown that yogurts with living *lattobacillus* permit lactose digestion in people with lactase deficit.

Minerals and vitamins

Milk fermentation allows the volatilization of calcium, magnesium, phosphor and oligo-elements increasing the minerals biological availability. It has been demonstrated that calcium and phosphor present in the yogurts are more available than the ones in the milk. A regular daily ingestion of yogurt is also associated with a significant increase of plasmatic calcium and a subsequent improved immune response function. Milk and fermented milk have the same vitamins concentration but during fermentation we have depletion of B12 and C vitamins and production of folic acid. All other vitamins don't appear to be significantly modified.

Conclusions

Nutritional aspects of fermented milks are related to the specific employed probiotic bacillus: the ingestion of specific quantity of these micro organisms has an important role on health. For this reason, fermented milks are identified as "functional food", characterized by a good nutritional value and the ability to improve the health of people.

Table 4. Nutritional composition of yogurt made with half skimmed milk (from Syndifrais, 1997)

		per 100 g	per 125 g
Proteins	g	4.3	5.4
Lipids	g	1.2	1.5
Carbohydrates	g	5.0	6.2
Kcal		48	60
KJoules		201	251
Calcium	mg	148	185
Phosphor	mg	114	142
Magnesium	mg	13	16.2
Vitamin B1	mg	0.04	0.05
Vitamin B2	mg	0.18	0.22
Vitamin PP	mg	0.11	0.14
Vitamin B5	mg	0.35	0.44
Vitamin B6	mg	0.04	0.05
Vitamin A	g	5	6.25
Vitamin E	mg	0.03	0.04
Vitamin C	mg	1	1.25