The health benefits of probiotics

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NUTRITOPICS

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Éditorial

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Society in Europe is characterized by the plentiful supply of food, even though it is still unevenly distributed. The fear of hunger has gradually been superceded by worries about food safety. This is why consumer demand is turning to products that make promises about health. Probiotics have had this aspect since the concept was first invented, and the first yogurts were supposed to increase longevity (Metchnikoff). This original situation created a discrepancy between the health dream and the reality of the products available, because very little research was in fact carried out to support these "health" claims. Increased consumer demand has generated the supply. The competition between the various products has led to a greater determination to provide increasingly reliable evidence that is recognized by the scientific world to support "health effects". The measures taken by the French *Agence Française de Sécurité Sanitaire des Aliments*, and more recently by the European Agency, have supported this shift, and probiotics now have to convince these authorities that their claims can be justified. The United States and Canada do not yet have these requirements.

A new scientific challenge is appearing in the area of research into probiotics and, more generally, into human diet; this concerns the influence of the food matrix. Are the beneficial effects on human health the same regardless of whether the probiotic is consumed in the form of fermented milk or a capsule? This is the whole difference between a food and a dietary complement, highlighted by the major SUVIMAX* study in Man.

The search for new strains of probiotics should also benefit in the years to come from methodological and conceptual advances in the field known as "integrative biology", "high-flow biology" or "transcriptomics, proteomics and metabolomics". These approaches will make it possible to study all the syntheses and regulatory processes that occur in a given medium, in particular in the digestive tract. This will enable scientists to elucidate the mechanisms of action of known probiotics, and to identify new concepts for a targeted panel of probiotics for tomorrow. These studies should, to an even greater extent than in the past, involve both publicly-funded research and private research.

** SUpplémentation en VItamines et Minéraux AntioXydants (vitamin and antioxidant mineral supplementation) a major French nutrition survey carried out between 1994 and 2003, involving 13,000 volunteers who took either a placebo or a combination of antioxidant vitamins and trace elements (http://www.suvimax.org).*

Report

What is a probiotic?

There is often a lot of confusion between probiotics, microorganisms and ferments. What distinguishes the first group from the other two? What daily foods are hidden behind these terms? Let's go back to the definitions.

summary

Summary

Probiotics are selected live microorganisms which, when they are ingested alive and in sufficient quantities, have a beneficial effect on health in addition to their conventional nutritional effects. They have been the subject of studies at the international level since the middle of the twentieth century.
Scientists are investigating the effects of these probiotics, and their combined effects, by trying to classify the beneficial effects of ingesting various strains and to elucidate their mechanisms of action within the digestive tract. At present, the methodological and ethical limitations of human studies make it difficult to understand the mechanisms of action of probiotics, and only partial explanations are available. Nevertheless, some possible benefits linked to the consumption of probiotics have already been suggested (better digestion of lactose, treatment for diarrhea-type disorders), and others are still being investigated (reduced risk of colon cancer, for example).

introduction

Introduction

The term "probiotic", which is derived from the Greek word for life, "*bios*", was invented in the middle of the last century as a result of observing the beneficial influence of certain microorganisms on the intestinal flora.
An everyday reality is hidden behind this scientific term: the fact that large numbers of live bacteria are consumed on a daily basis in fermented products. Yogurts, for example, contain some 10^7 to 10^8 *Lactobacillus bulgaricus* and *Streptococcus thermophilus* per gram of food.
Apart from the value of these species in the manufacture and storage of these fermented milks, ever larger numbers of studies are increasingly reporting wide-ranging health benefits for consumers.

consensus

Definitions of microorganisms, ferments and probiotics

The term **microorganism** is a generic term, and refers to the size of the organism (it is only visible under the microscope). Many consumers, since Pasteur, view microorganisms ("microbes") as vectors of diseases, but pathogenic microorganisms are relatively rare; most microorganisms are in fact harmless, or even beneficial. Probiotics are beneficial microorganisms.

The term **ferment** is used to designate a microorganism used for technological purposes: by way of example, we could mention dairy fermentation, or alcohol fermentation. This ferment is sometimes destroyed by heat treatment after it has produced its effect: this is what happens in the case of hard cheeses which have been cooked, sauerkraut, or heat-treated fermented milks, which make excellent deserts, but which have lost some of the health benefits provided by their ferments, which will have been destroyed by the heat treatment.
Most probiotics are ferments that are used in fermentation processes.

The term **probiotic** is used to designate a microorganism that provides a health benefit. Many other definitions of probiotics have been given, but in the end they are all very similar to each other. The one generally used at present was proposed by the FAO in 2002: **Live microorganisms, which when administered in adequate amounts confer a health benefit on the host.**

For the sake of simplicity, a food containing a probiotic is commonly referred to as a "probiotic food", and sometimes just as a "probiotic".
Yogurt is a probiotic food. To distinguish themselves from yogurts, the fermented milks launched onto the market in recent years have often tried to claim the name "probiotic" for themselves. In fact, yogurt is the archetypical probiotic food, because it meets all the criteria of a probiotic.
By definition, fermented milks containing killed microorganisms cannot be probiotic foods.

taxonomy

Some examples of probiotics

The probiotics that have been traditionally most often used are the lactic bacteria of the **genera** *Lactobacillus* and *Streptococcus*, plus the *Bifidobacterium* which are not strictly speaking lactic bacteria. Let us recall that lactic bacteria convert the sugar, or lactose, in milk into lactic acid, and this chemical reaction allows them to produce energy despite their having no respiratory chain.
According to the Codex Alimentarius standard adopted in Rome in 2003, **yogurt** contains exclusively organisms of the species *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in symbiosis, that are live and abundant. Fermented milks can contain the yogurt symbiosis and/or other species, such as *Lactobacillus casei, L. rhamnosus, Bifidobacterium breve,* or *B. bifidum*…
Different **species and sub-species** can be distinguished within a genus. Within a given species there may be a multitude of **strains**, characterized by a code or brand name, referring to a collection or to the Manufacturer's brand.

Genus	Species	Strains *(exemples)*
Lactobacillus	*L. bulgaricus*	Reference symbiosis S85
	L. casei	DN-114 001, ATCC334, YIT 9018
	L. rhamnosus	GG, ATCC53103
	L. johnsonii	La-1
	L. plantarum	Lp299v, WCFS1…
Streptococcus	*S. thermophilus*	S 85...
Bifidobacterium	*B. animalis*	DN-173 010
	B. breve	NCIMB8807
	B. longum	NCC2705, DJ010A…

What are prebiotics?

Probiotics must be distinguished from **prebiotics**, a term used to designate not microorganisms, but simple molecules, often very small sugars, such as the fructo- and galacto-oligosaccharides, but also fiber, inulin, lactulose, polyols, etc. The prebiotics represent a source of energy that can be metabolized by the intestinal or probiotic microflora (1;2). These molecules may be added to a food or produced *in situ*, like the oligosaccharides, which are produced by certain bacteria during lactic fermentation.

a probiotic is a live micro-organism used for fermentation, and which confers a health benefit

Where do probiotics come from?

A question about where probiotics come from may seem surprising, and yet, paradoxically, all consumers ask themselves about this ... and they also ask their GP. It is a bit like the eternal chicken and egg question; people usually want to know whether probiotics developed before or after their hosts.

history

The definition of probiotics has changed as a result of scientific discoveries

It was most probably Ferdinand Vergin (3) who introduced the term "probiotic" in 1954, when in an article entitled "Anti- und Probiotika" he compared the harmful effects of antibiotics and other anti-microbial substances on the flora, with the beneficial ("Probiotika") effects of the beneficial bacteria. A few years later, in 1965, Lilly and Stillwell described probiotics as microorganisms that stimulate the growth of other microorganisms (2).

Subsequently, the definition of a probiotic was extended to include the idea of "a beneficial effect on growth by an effect on the host's flora"; this definition given by Füller (4) was based on studies carried out in animals. This definition was difficult to apply, because of the lack of any appropriate markers of the effect on the flora. It was also a very restrictive definition, because microorganisms sometimes act by means other than their effect on the flora. However, it indicated that there was already a search for a mechanism of action, and the flora was logically the most obvious site of action.

History therefore implies that the current definition could still undergo further changes, because there are still many fields of research seeking to explore and explain the effects of probiotics: their role in terms of the regulation and interaction with the intestinal flora, factors responsible for diversity in Man, the factors that determine their establishment and maintenance, the contribution of each constituent and of the community as a whole to the health benefit etc. (5).

> Find out about the history of the definitions of the word "probiotic" on our website: www.danonevitapole.com

evolution

Bacteria are not of human origin

Some consumers want to believe that the only "good" probiotics are of human origin. Yet, two facts actually contradict this idea. Firstly, bacteria were present on the Earth for millions of years before Man appeared. Secondly, the digestive tract is sterile when a baby is born, but it soon proves to be an excellent ecological niche, containing little oxygen but plenty of nutrients. Consequently, an initial digestive flora is installed very soon after birth, originating from the immediate surrounding world, and then changes to become highly complex in adults, with more than 10^{11} bacteria, belonging to more than 400 different species, per gram of stools.
Bacteria cannot therefore strictly speaking be of "human origin". However, some of them are better able than others to survive and therefore to become established in Man. This group has colonized the digestive tract, and the process of selection has retained the best-adapted strains. This is why the human digestive flora has a fairly stable composition in terms of the genera it contains. This selection process continues even within each individual. The human species in general is inhabited by the same dominant genera, but the species present can differ from one individual to another.

[Probiotics are not of human origin]

adaptation

Ecology of the digestive tract

It appears that within a living being bacteria have adapted to the conditions prevailing in each segment of the digestive tract, and have recreated and maintained an anaerobic atmosphere (6). Since the environmental conditions change as transit through the intestine progresses, bacterial populations are specific to a specific segment of the digestive tract. In healthy subjects, the stomach and duodenum are only lightly colonized (10^1 to 10^4 cells/ml), because the pH conditions and changes are unfavorable. In addition, this transit leaves the bacteria very little time to attach themselves. In contrast, the bacterial population is higher in the more distal portions of the small intestine (10^4 to 10^8 cells/ml) and, to an even more marked extent, in the colon where maximum concentrations can reach 10^{12} cells/g, mainly in the caecum which is the site of intense fermentation.
Furthermore, it has been observed that the species present vary depending on the animal and the residues of its diet. For example, herbivores have a flora containing high levels of cellulolytic bacteria, such as *Clostridium*, *Ruminococcus* and *Fibrobacter* (7), whereas Man has a flora containing large amounts of amylolytic bacteria, such as *Bacteroides* (8;9).

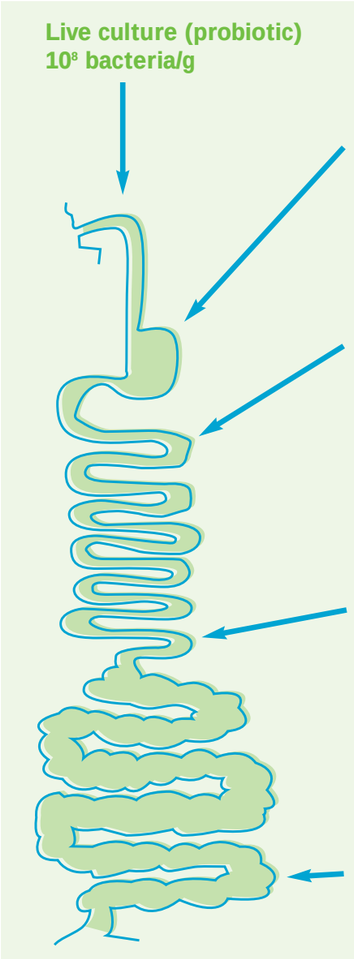


Figure 1
The flora at the different levels of the digestive tract (10)

The specific characteristics of probiotics

Probiotics display specific characteristics both with regard to their mechanical properties, notably their ability to attach themselves to the intestinal mucosa, and their colonization and survival activity. These are the properties that underlie the models used to explain their health effects.

A major challenge facing scientific research is that of identifying probiotic strains, since this is essential if a given effect is to be attributed to a particular strain, and also to make it possible to distinguish between the probiotics being investigated and the resident intestinal flora. The methods used to detect probiotics have changed considerably in recent years. There are now several different methods available, including the following:

- the use of selective culture media;
- the use of **polyclonal antibodies**, immunological markers that reveal the presence of specific strains, such as the probiotic *Bifidobacterium animalis* DN-173 010 (11);
- **molecular biology techniques**, based on the detection of DNA using the FISH method (*Fluorescent In Situ Hybridization*), which is able to detect specific genera and sometimes even specific species of bacteria by using complementary fluorescent probes for RNAr 16S. Another more sensitive approach involves the PCR method (*Polymerase Chain Reaction*) or qPCR (*Quantitative Polymerase Chain Reaction*) and is able to identify individual species (12).

Probiotics must remain active if they are to produce a health benefit

viability

The difference between survival and activity

Although probiotics that are eaten while they are alive are not able to establish themselves in the digestive tract for any length of time, they must be able, as their definition demands, to **remain viable** if they are to produce a health benefit.

To date, several probiotics have been shown to survive in Man, such as *Bifidobacterium*, for example (13-15).

Strictly speaking, activity has only been demonstrated in animals. A recent method is based on the genetic modification of strains by introducing marker genes that make it possible to estimate the survival and metabolic status of a specific strain. This approach has been applied to strain DN-114 001 of *L. casei* (16). Another study involving *Streptococcus thermophilus* has shown that the bacterial cells activate their metabolism and hydrolyze lactose in the intestine (17).
The purpose of these new methods is to make it possible to demonstrate the activity of probiotics in Man.

Figure 2 ***(on the right)***
An example of colonization kinetics (20)

Colonization kinetics after the daily ingestion for one week of 300 ml of milk fermented with a rifampicin-resistant variant of *L. casei* DN-114 001.
In this study, the volunteers consumed the same amount of the product daily for a week. To determine the survival of the strain in the feces, the stools were collected before the first time the product was consumed (S1), 4 days later (S2), at the end of the consumption period (S3) and then 3 and 7 days after stopping the consumption (S4 and S5).

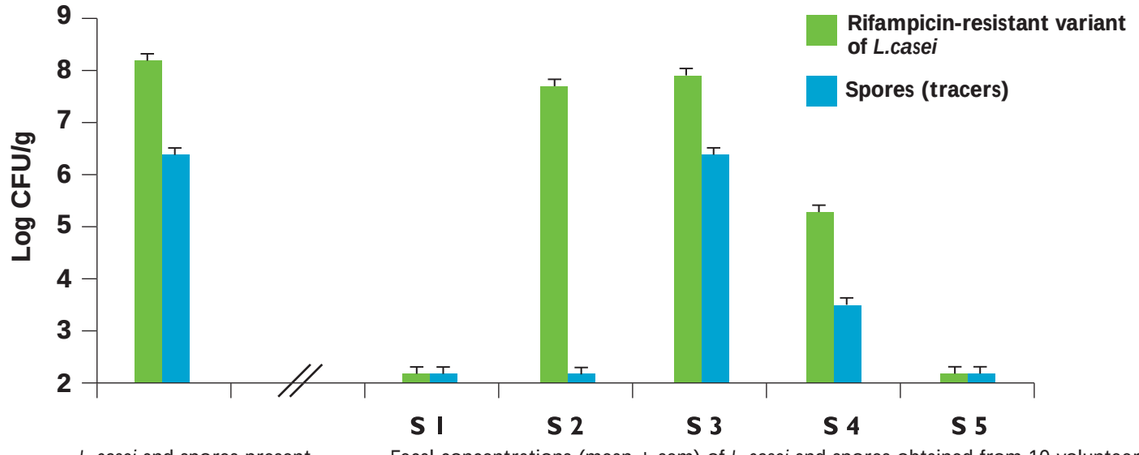
transit

A transient presence in the digestive tract

In French, the term **"colonisation"** is generally used to refer to the ability of microorganisms to reach the alimentary canal, and then to establish themselves and remain there. In English, however, the term is used to describe a "colonizing" population that arrives, remains a few days and then moves on.
In this second sense, which is the one we shall be using here, colonization does not imply any major disruption of the flora; it is a **reversible and transient** phenomenon. The kinetic data make it clear that after 300 ml of milk fermented with a rifampicin-resistant variant of *L. casei* DN-114 001 has been ingested daily for one week, a stable population is established within four days, but then disappears within three to five days after the subject stops consuming the fermented milk (see Figure 2).

Probiotics sometimes simply "nest" in the mucus on which they feed. Some bacteria therefore have a **chemotropic mechanism** for the mucus of the digestive tract, where they find the sugars that constitute a competitive source of energy. They therefore develop beside the mucosa, which is where sugar levels are highest. This location facilitates their interactions with the intestinal cell. Strictly speaking this can no longer be called "adhesion". The part of the flora "nested" in the mucus is rather inaccessible in Man.

Strictly speaking, the adhesion of a microorganism, and in particular that of pathogens, to the intestinal wall is linked to the presence of **adhesins**, molecular structures that are able to recognize complementary structures or specific receptors on the membrane of the cells of the mucosa, to which they can attach themselves (18).
The ability of various strains of *Lactobacillus* to adhere to the epithelial cells, and thus to inhibit the adherence of pathogens such as *Escherichia coli* or *Salmonella typhimurium*, is probably linked to steric obstruction of the enterocytic receptors for pathogens (19).
This hypothesis of competition between pathogenic microorganisms and probiotics for a limited number of binding sites is generally accepted, even though it has never actually been confirmed *in vivo*.



L. casei and spores present in the fermented milk

Fecal concentrations (mean ± sem) of *L. casei* and spores obtained from 10 volunteers after they had ingested fermented milk according to the consumption schedule

The effects on health

The beneficial effects of probiotics consist of either reducing one or more risk factors for disease, or of improving bodily functions. There is increasing evidence that they do indeed produce these effects (21). In 1999, the team of Naidu (22) noted 143 clinical trials with probiotics carried out between 1961 and 1998, involving more than 7500 subjects, none of whom reported any side effects. In general, very few harmful or unpleasant side effects have been reported following the taking of probiotics.

Each species, even each strain, offers a greater or lesser health benefit. These effects include those listed below (see table):

digestion

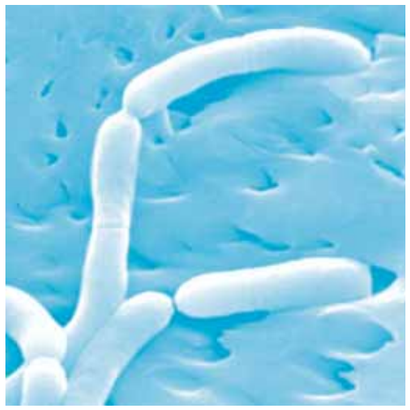
Improved absorption and regulation of transit

• **Lactose intolerance:** studies have shown that yogurt, because of the live microorganisms it contains, improves the digestion of lactose in individuals suffering from lactose malabsorption and relieves their gastrointestinal symptoms. These benefits are considerably reduced if the product is subjected to heat treatment.

• **Transit:** fermented milks improve intestinal transit in general (23), and more particularly in people whose transit is sluggish (24-26).

• **Diarrhea:** fermented milks regulate diarrhea in infants (27-30) and bacterial diarrhea (31).

They improve **the tolerability of antibiotic treatments** by reducing diarrhea following medication (32).



Lactobacillus bulgaricus

The effects of prebiotics

Very considerable attention has been paid to the observation that breast-fed babies have a flora in which bifidobacteria predominate. This native infantile flora is maintained by breast feeding, partly as a result of the galacto-oligo-saccharides contained in the breast milk (33). This interaction between the flora and human breast milk has highlighted the value of the bifidogenic effects of dietary supplements.

It has also been shown that the oligofructoses and oligosaccharides have a significant effect on the population of the endoluminal flora by stimulating the bifidobacteria in particular (34).

The most important effects of the prebiotics may involve boosting the resistance towards pathogens and so preventing diarrhea. However, for the moment, this effect has not been convincingly demonstrated in either adults or children (35).

Streptococcus thermophilus

The effects reported and their putative mechanisms (36)

Benefit	Function	Proposed mechanism	Reference
Digestive comfort	Irritable bowel syndrome, symptoms affecting the gastrointestinal tract in general (constipation, non-pathogenic diarrhea, distension, flatulence, cramp, halitosis of a digestive cause)	Change in the populations or activities of the intestinal microflora	37
	Lactose intolerance	Delivery of microbial lactase to the small intestine	38
Defense	Allergy (atopical eczema, allergy to milk, rheumatoid arthritis)	Translocation, barrier effect	39 ; 40
	Cariogenicity	Changes in the populations, activity of the oral microflora or its ability to adhere to the teeth	41
	Carcinogenicity, mutagenicity, tumor	Absorption of the mutagen, stimulation of the immune system, inhibition of carcinogen production by the intestinal microflora	42 ; 43
	Diarrhea linked to antibiotics, diarrhea caused by *Rotavirus*, colitis caused by C.*difficile*, nosocomial diarrhea	Competitive exclusion, translocation/barrier effect, immune response promoted	44
	Helicobacter pylori	Antipathogenic activity	45
	Immunomodulation (immune status, vaccinal response)	Interaction with the immune cells or cell receptors leading to an increase in the phagocytic activity of the white cells, increasing IgA levels after exposure to the antigen, increasing the proliferation of intra-epithelial leukocytes, regulating the Th1/Th2 ratio, induction of cytokine synthesis	46
	Intestinal inflammation, ulcerative colitis, Crohn's disease, pouchitis	Immune response downregulated	47
	Excessive intestine bacterial growth	Antimicrobial activity, competitive exclusion	48
	Vaginosis, urinary infections	Antipathogenic activity, competitive exclusion	49
Others	Lowering of blood cholesterol	Deconjugation of the bile acids	50
	Endotoxemia combined with cirrhosis	Inhibition of the production of endotoxins by the intestinal microflora	51
	Hypertension	Cellular constituents or peptides derived from fermentation acting as inhibitors of ACE (angiotensin-converting enzyme)	52
	Renal calculi	Changes in the digestive flora influencing the breakdown of oxalate	53

defense

Effects on the risk factors for diarrhea and colon cancer

The effects on the risk factors for diarrhea and colon cancer involve the neutralization of the attack and an improvement of repair capacities.

Effect on the risk factors for diarrhea:

The defense effect of probiotics involves an effect on the flora, an effect on the wall and the mucus, and an effect on the immune system. The health value of probiotics with regard to these three points has been demonstrated, for example, both in preventing travelers' diarrhea (54) and the diarrhea linked to taking antibiotics (55). It is unlikely that the explanation for these effects can be simply attributed to the massive arrival of probiotics that compete with the partially pathogenic flora already present (56). Many studies now suggest that, in addition to this modulation of the composition of the intestinal flora, some probiotics also affect the immune response of the lymphoid tissue associated with the gastroalimentary tract (GALT) (57-59), although the mechanisms of this immunomodulation remain to be elucidated.
This health effect has been demonstrated in healthy individuals: thus the consumption of *L. rhamnosus* GG for thirty weeks by children between 1 and 6 years of age has been monitored. The authors have shown a reduction in the numbers of days of absence due to illness and a reduction in the use of antibiotics prescribed in a context of respiratory illness (60).

Effect on the risk factors for colon cancer:

The work of an Italian team at the University of Perugia (61) has now provided tangible evidence of the efficacy of lactic bacteria against cancer, in animals at least. This team has carried out *in-vitro* tests of the ability of various strains of lactic bacteria, obtained from commercial dairy products, to inhibit the genotoxic effects of a potent carcinogen, 4-nitroquinoline-1-oxide, and has shown that some probiotic strains have a powerful inhibitory effect against this carcinogen. This effect was found to be both species- and strain-dependent.
This effect of reducing the risks of colon cancer has been demonstrated by many other studies, some of them carried out *in vivo* in mice with a human intestinal flora (62) and in the rat, in the prevention of the harmful effects caused by some carcinogens on the DNA of the colon cells (63).

Various hypotheses have been advanced concerning possible mechanisms that could account for this protective role: probiotics could either reduce cell proliferation, or promote the apoptosis of diseased cells (64); even though they sometimes actually restrict apoptosis in healthy cells (65).

other functions

Effects that have already been identified and new avenues of research

Many other beneficial effects of probiotics in healthy subjects have either been demonstrated or have been investigated, but so far no consensus has been reached about them:

- stimulation by *Lactobacillus* GG of the production of nitrogen oxide NO, a molecule that has a range of effects on the immune and vascular systems (66;67);
- stimulation of the chemotaxis of macrophages, making it possible to eliminate pathogens from their migration site (68);
- the effect on blood cholesterol levels is still very controversial. For instance, one study has reported a reduction of the total blood cholesterol in women who were eating 3 yogurts per day, but not in men (69). Another study has reported a significant lowering of blood cholesterol after the consumption of yogurt or heat-treated fermented milk, but much less effect with unprocessed milk (70). Other studies report no such effect (71;72). At the very least, no study has reported any hypercholesterolemic effect, which makes yogurt a promising vector for anti-cholesterol ingredients.

[the health effects depend on the species or even the strain of the probiotic]

Specific conditions required for the expression of the beneficial effects

Many of the health effects of the probiotics were identified during studies carried out using yogurts and fermented milks. These effects are not always confirmed when using heat-treated products that contain no live flora. Furthermore, it appears that the vector of the probiotic, in this case the dairy product, plays a crucial role.

medium

An effect of the vector?

The survival of the probiotic depends on its intrinsic resistance, but also on the host and the ingestion vector (73).

The gastric acidity constitutes a major defense mechanism. Pedrosa has demonstrated that the survival of *L. gasseri* ADH is high in subjects with hypochlorhydria, and that the resistance towards acids varies considerably depending on the probiotic (74). Pochart observed that the concentration of the viable yogurt bacteria reaching the duodenum after ingesting yogurt containing 10^7 CFU/ml, was no more than 10^5 CFU/ml (CFU, colony forming unit) (75). Pettersson reports that 10^8 bacteria of a yogurt reached the ileum in a viable state (76). Some bacteria, such as *Bifidobacterium* and *L. plantarum* NCIB8826 demonstrate excellent resistance throughout the digestive tract, and 10^8 bacteria are found in the feces (73).

Another selective factor, in addition to gastric acid, are the **bile salts**: for instance *Lactococcus lactis* MG1363 and *L. fermentum* KLD have very limited survival in the ileum. Other authors have also highlighted this bactericidal effect, which could be linked to increased membrane permeability as a result of a detergent effect of the bile salts on the cell membranes (77).
Finally some experimental data suggest that the **pancreatic secretions** may have a bactericidal role against some strains. An *in-vitro* study has also been carried out in which a suspension of probiotic cells was first exposed to simulated gastric juice and then to simulated duodenal juice, in order to mimic gastrointestinal transit. The effects of this procedure varied considerably from one strain to another, some of them being resistant and others highly susceptible (78).

The **vector** plays a crucial role because these three "barriers" have to be crossed. It has been clearly demonstrated that dairy products have a high buffering potential (79) in the stomach, which has the effect of protecting the bacteria (71;72;80). Thus, it has been shown in vivo that the presence of milk increases the resistance of *L. acidophilus* ADH to gastric acidity (2 h in the presence of milk versus only 40 to 60 minutes in its absence). The numbers of *L. bulgaricus* and *S. thermophilus* organisms that survive are also greater when they are ingested in yogurt (75). Saxelin *et al.* have demonstrated that the survival of *Lactobacillus* GG was the same regardless of whether it was ingested in capsules that dissolve in the duodenum or in fermented milk (81;82). Although there was no dose-related difference for the consumers of the capsules for doses ingested of between 1.10^9 CFU/day to 8.10^9 CFU/day, in contrast it was found that the concentration of lactobacilli in the feces did depend on the dose of bacteria ingested daily in the fermented milk (dose of $2.1x10^9$ CFU/day versus $1.2x\ 10^{10}$ CFU/day).
The survival of the probiotic therefore depends on the medium in which it has fermented. Its activity, and therefore its health effect, also depends on this: for example, a study of a panel of 10 probiotic strains has revealed differences in the antimutagenicity in the Ames test, depending on whether the strain has been fermented on a synthetic medium or on milk (83).

quantity

Is there a dose effect?

The bacterial population in transit must also be able to use its own enzymes to produce enough of the molecules that act on the host or at the site of action for the health benefit to be perceptible. This assumes that there are more than a certain number of viable cells and this number has been variously assessed by different authors as between $5x10^6$ and 10^8 per gram of the digestive contents. For instance, studies of *L. rhamnosus* GG (60) have shown that the beneficial effects are observed from doses of about 10^7 to 10^8 CFU.
Below this concentration, the potential effects of the microorganisms in transit will not be detected (84).

Probiotic consumption in terms of figures

Lactic acid bacteria are consumed in very large quantities, above all in the form of fermented products. According to a bulletin published by the International Dairy Federation (IDF- FIL), the annual consumption of fermented products is 17.6 kg per person in Europe i.e. some 6.6 billion kg of fermented milk! Since the average bacterium content of fermented milk is about 10^8 bacteria per gram, this total corresponds to 6.6×10^{20} lactic bacteria… or 2 640 metric tonnes of lactic bacterial cells (each bacterium weighing about $4x10^{-12}$ g)!

Pathways of actions

The complexity of the digestive microflora is now recognized: colonization begins at birth, and then continues throughout life, leading to the establishment of a flora consisting of more than 400 different species (85). We are only just beginning to learn about this digestive flora, and our understanding of our digestive ecosystem should develop considerably with the introduction of molecular technologies and miniaturized local investigations. Meanwhile, many questions remain and we can only provide tentative explanations.

population

Effect on the flora

• its composition:

the equilibrium of the intestinal flora results from the interactions between microbes within the ecosystem in the form of competitions (for nutritive substrates or adhesion sites) and of changes to the ecosystem brought about by the products of bacterial metabolism (pH, organic acids, bacteriocins) (71;72). The consumption of probiotics therefore influences the composition of the intestinal flora.

For example, the consumption of *L. casei* increases the level of lactobacilli in children (86).

• its function:

As probiotics affect the composition of the intestinal flora, they can also affect its function.

Several possible mechanisms have been suggested that could account for the effect of probiotics on intestinal disorders and diarrhea, notably the production by some lactic bacteria of antimicrobial substances (hydrogen peroxide, bacteriocins and organic acids for example) which have a damaging effect on the growth of pathogens, and modulate the function of the immune system.

The probiotics could influence the ability of the flora to hydrolyze the glucose bonds; this ability has a limiting effect, for instance, on the uptake of the isoflavones of soy (87). Some lactic bacteria are able to reduce the activity of some enzymes of the flora, such as ß-glucuronidase, ß-glucosidase, azoreductase and nitroreductase, which are responsible for converting procarcinogenic substances into carcinogens. This has been demonstrated in animal species and in Man (71;72;88).

communication

Effect on the intestinal cells

It has to be admitted that the molecular details of the interactions between the flora and the host are still poorly understood. Overall, this is known to involve cross-talk: the bacterium "talks" to the intestinal cell and in so doing affects its function (89).

Some microbial signals have been identified, including microbial formyl peptides such as formyl-methionyl-leucyl-phenylalanine (90), liposaccharides, the glycopeptides that make up the cell wall and nucleotides. The innate immune response of the host distinguishes between the signals of the pathogens and those of the beneficial bacteria by means of specific receptors. The immune cells seem to use various receptors that recognize different specific points in the bacterial system: lipoproteins, glycopeptides, flagellin, microbial DNA. The microbial DNA, such as the oligonucleotides, contain non-methylated dinucleotides that stimulate the lymphocytes, whereas eukaryotic DNA and the methylated oligonucleotides do not. Rachmilewitz *et al.* have recently demonstrated that the beneficial effects of a probiotic on a population of mice suffering from chemically-induced colitis are linked to the DNA of bacteria, since the non-methylated DNA of this bacterial strain was sufficient to induce a beneficial effect (91).

Recent studies have also shown that the consumption of probiotics limits the increase in intestinal permeability observed in rats after infection with *E. coli* (92).

The Mac/Gac concept

The interactions of bacteria with the digestive tract have a major impact on the host, but also on the other bacterial populations. Studies of these interactions have benefited from a sterile animal model and the Mac/Gac concept (93;94). The microflora-associated characteristics (Mac) correspond to any structure or physiological, biochemical, immunological or anatomical function of the microorganism that is influenced by the presence of other microorganisms. This is done by comparing them to the corresponding structures and functions in sterile animals (Gac, germ-free animal characteristics). It emerges from these studies that the intestinal microflora is not essential for survival, but that the flora does have observable effects on:

- the anatomy and histology of the digestive tract (the sterile animals have a dilated caecum with a thin mucosa)
- the rate of transit (slower in the sterile animals)
- the biochemistry of the large intestine (some enzymes are missing …)
- an effect on immune activity (numerous aspects of macrophage function, less CD4+…)

peptides

Active metabolites

So far, two major categories of active peptides have been identified:

• the peptides resulting from the hydrolysis of certain proteins

According to recent studies (95), the two probiotic strains *L. helveticus* R211 and R389 are able to hydrolyze casein and so generate peptides that inhibit ACE (*Angiotensin Converting Enzyme*). This production of peptides by a mechanism other than that of intestinal digestion may, according to the same team, be linked to the lowering of the blood pressure in the hypertensive rat (52;96); subsequent studies have also confirmed this effect in Man (97).

The biopeptides produced by *Lactobacillus helveticus* may be involved in the immunomodulating effects of this probiotic: increasing the amount of secretory IgA in the bronchi and intestine. This effect has not been detected for a non-proteolytic variant of the same strain (98).

• the bacteriocins synthesized by the microorganism

The bacteriocins are antibacterial substances produced by some groups of microorganisms, including probiotics and lactic acid bacteria. They are usually considered to be distinct from antibiotic substances, and they inhibit the growth of unwanted microorganisms in foods and dairy products.

conclusion

Conclusion

Obvious global effects have therefore been identified in Man, involving digestive comfort with reduced lactose intolerance; defense mechanisms with less diarrhea for example and hypertension, renal calculi etc. …

The studies carried out have also highlighted that some mechanisms are activated after ingesting probiotics, particularly at the level of the flora and its metabolism.

Scientists now have models that demonstrate some of these effects, such as the mouse with a human flora, and also studies that can be carried out *ex vivo* and *in vitro*. They also have effective tools, such as mucus analysis, or biotechnological methods. These studies and tools open the way to the discovery of further benefits.

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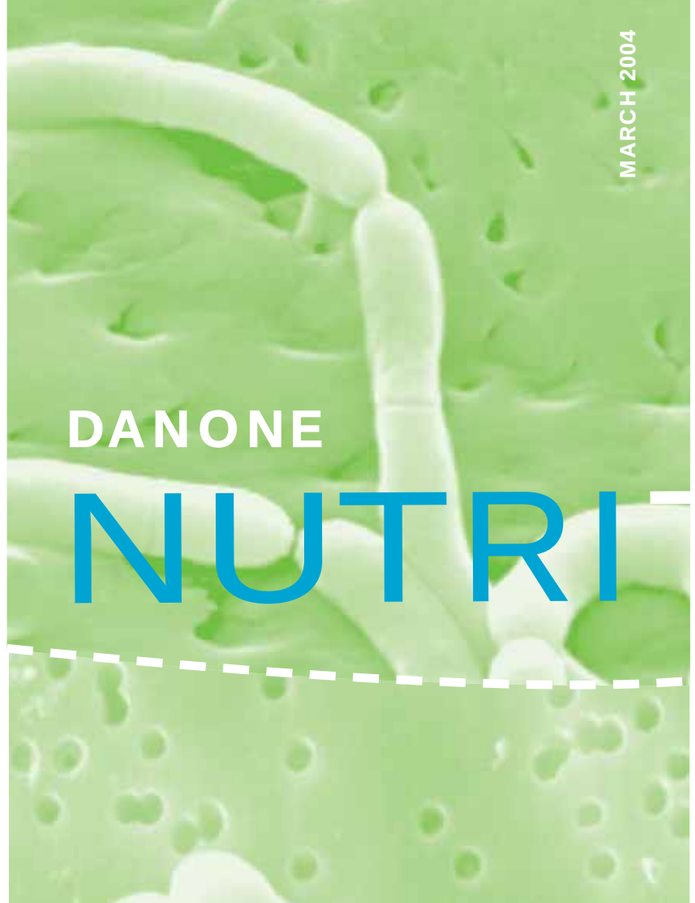

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A short history of the definition of the term "PROBIOTICS"

1954 First reference to the positive effects (« Probiotika ») of beneficial bacteria.
Vergin F. Anti- und Probiotika. *Hippokrates* 1954;25:16-119.

1965 **A substance secreted by one microorganism which stimulates the growth of another**.
Life nourishes life: "pro-biotic".
Lilly D.M., Stillwell R.H. Probiotics: Growth promoting factors produced by microorganisms. *Science* 1965;147**:**747-748.

1971 **Tissue extracts which stimulate microbial growth**.
Sperti G.S. *Probiotics*. Avi Publishing Co, Westpoint, Connecticut, USA. 121 p.

1974 **Organisms and substances that contribute to intestinal microbial balance**.
Parker R.B. Probiotics, the other half of the antibiotic story. *Anim. Nutr.Health* 1974;29:4-8.

1992 **Live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance**.
Füller R. Probiotics: the scientific basis. Chapman and Hall Ltd, London, p.1. 1992.

1992 **Viable mono- or mixed culture of live microorganisms which, applied to animals or Man, have a beneficial effect on the host by improving the properties of the indigenous microflora.**
Havenaar R., Huis int'Veld J.H.J. Probiotics: a general view. *In Lactic Acid Bacteria in Health and Disease*, vol. 1. Edited by BJB Wood. Elsevier Applied Science Publishers, London, pp. 151-170.

1996 **Live microbial culture or cultured dairy product which beneficially influences the health and nutrition of the host**.
Salminen S. Uniqueness of probiotic strains. *IDF Nutrition Newsletter* 1995;5(145):18.

1996 **Food supplement, consisting of live microorganisms, which beneficially influences the host (animal or human).**
«…ein Lebensmittelsupplement in Form lebender Mikroorganismen, das den Wirtsorganismus (Tier oder Mensch) günstig beeinflusst.»
De Vrese M. Probiotische Milchprodukte. *Deutsche Milchwirtschaft* 1996;5(145):18-19.

1996 **Living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition**.
Schaafsma G. Significance of probiotics and prebiotics in human diets. In 21 st International congress on microbial ecology and disease, LABIP consensus, SOMED, October 28-30, 1996, Paris: Institut Pasteur, abstract 117, p.38.
Also cited in: State of the art concerning probiotic strains in milk products. *IDF Nutrition Newsletter* 1996;5(145):23-24.

1997 **Live microorganisms which positively influence the health of the host (animal or Man), by means of a better balance in the intestinal flora**.
«Lebende Mikroorganismen, welche die Gesundheit des Wirts(tieres) günstig beeinflussen, indem sie das Gleichgewicht der Intestinalflora verbessern»
and in a more general manner: **Live microorganisms which positively influence the host (animal or Man), beyond basic nutritional properties of food.**
«Lebende Mikroorganismen, die den Wirtsorganismus (Tier oder Mensch) in einer über die Eigenschaft des Lebensmittels als Nährstofflieferant hinausgehenden Weise positiv beeinflussen».
Schrezenmeier J. Gesundheitsrelevante Aspekte von Probiotika - Mythos oder Realität? *Die Molkereizeitung Welt der Milch* 1997;51:956-958.

1998 **Living microorganisms which favorably influence the health of a host by improving the indigenous microflora.**
Schrezenmeier J. Actual knowledge and prospection. In *2nd Symposium Danone, Fermented food, fermentation and intestinal flora. Immunity and probiotics*. Bonn, October 30, 1998: Abstract.

1998 **Live microbial foods which have a beneficial effect on the intestinal microbial balance**.
Zoppi G. Probiotici, prebiotici, sinbiotici ed eubiotici (Probiotics, prebiotics,synbiotics and eubiotics). *Pediatr. Med. Chir.* 1998;20(1):13-17.

1998 **Living microbial food ingredient that is beneficial to health**.
Diplock A.T. *et al*. *Scientific concepts of functional foods in Europe*. Consensus document (FF/27/DE98). Brussels, ILSI Europe, p.50.
Br.J.Nutr. 1999 ;81(4) Suppl.1.

2002 **Live microorganisms which when administered in adequate amounts confer a health benefit on the host.**
FAO Definition 2002.
Guidelines for the Evaluation of Probiotics in Food, Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London Ontario, Canada, April 30 and May 1, 2002,
http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf