Yoghurts

Yoghurt, the product of milk fermentation by Lactobacillus delbrueckii ssp. bulgaricus (L. bulgaricus) and Streptococcus thermophilus, has a long standing reputation as a nutritious, natural and safe component of a healthy diet, and is at the basis of the concept of probiotics. It is one of the economically most important dairy fermentation products, while the market for derived probiotic products containing additional bacteria such as Lactobacillus casei is rapidly expanding.

With the recent publication of the complete genome sequence of L. bulgaricus, and the sequence of S. thermophilus which was published two years ago, we now dispose of a wealth of information to build on when studying yoghurt bacteria and the interactions between them and with their environment.

The analysis of the L. bulgaricus genome tells us that it is in a state of rapid evolution, in what appears to be an adaptation to the lactose and protein rich milk environment. An extremely high number of pseudogenes and incomplete metabolic pathways indicate that this bacterium has lost a good deal of its carbohydrate metabolism and amino acid biosynthesis capacities to capitalize on milk resources.

L. bulgaricus is a member of the acidophilus complex, a group of closely related lactobacilli that contains a remarkable number of species with alleged probiotic properties (L. acidophilus, L. johnsonii, L. gasseri). This group was formerly known as the delbrueckii group, but received its present name when the L. delbrueckii GC content (50%) appeared to be very different from that of the other members of the group (35%). The L. bulgaricus genome sequence reveals that the difference is primarily found at the third position of codons, where the GC content is extraordinarily high when compared to other bacteria. This observation suggests that L. bulgaricus is evolving toward a higher GC content. The atypical GC content thus seems to reflect a recent development and indicates a different future rather than a different history.

Although phenotypically L. acidophilus and L. johnsonii look much more alike than either of them and L. bulgaricus, 16S rRNA and now also genome wide protein based phylogeny unambiguously show that L. acidophilus is closer related to L. bulgaricus than to L. johnsonii. Extensive horizontal gene transfer appears to have occurred between L. acidophilus and L. johnsonii, which may explain (part of) their resemblance.

L. bulgaricus thus seems closer to the other members of the acidophilus complex than once thought, reviving the controversial question of whether this bacterium can be considered probiotic or not. While L. bulgaricus has already been implicated in the improvement of lactose tolerance, the present genome sequence suggests that strain ATCC11842 may be able to produce folate, an essential B vitamin. Both properties would meet the definition of probiotics proposed by an FAO workgroup which states that they are "live microorganisms which when administered in adequate amounts confer a health benefit on the host".

Regarding the state of ongoing evolution observed in this bacterium, important differences may be expected between different strains and as a matter of fact have been observed when measuring for example acid resistance. The same may apply to probiotic properties. Strain ATCC11842 does for example contain a partial bile salt hydrolase gene, a complete copy of which may be present in other strains and affect survival in the human gastrointestinal tract. Only further research can provide the answers.


The fate of probiotics in the digestive tract

The survival of probiotics as they journey through the digestive tract is often considered as a key criterion in guaranteeing their beneficial effect on human health, at least for a certain number of them. Two independent publications have evaluated the survival of different probiotics.

The first study (1) measured the persistence of yoghurt starters in the faeces of adults who had consumed a commercial yoghurt during one week. The experimental protocol required those enrolled to abstain from fresh milk products and yoghurt for the 2 weeks prior to inclusion. The following week, they were required to consume 2 yoghurts per day (5x10^13 CFU of Streptococcus thermophilus and 6x10^10 CFU of Lactobacillus bulgaricus). These two bacteria were identified in faecal samples using a selective medium. This medium supports the growth of clearly distinguishable colonies and permits to clearly distinguish the pink halos of streptococci from the yellow ones of lactobacilli. In the next step, the specific strains of streptococci and lactobacilli were identified by molecular tools. Although L. bulgaricus was collected live from all 10 subjects enrolled in the study, S. thermophilus was only collected live from one of them.

The second study (2) had as its objective to assess the viability of Lactobacillus casei DN-114 001 both in the ileum and in the faeces. The total quantity of L. casei DN-114 001 collected in the ileum after 8 hours was on average 1.6x10^10 CFU, which corresponds to an average live lactobacillus retrieval rate of 3.6% compared to the quantity consumed (300 mL of fermented milk containing approximately 10^10 CFU/mL absorbed in a single intake).

In the faeces, the maximum quantity of L. casei DN-114 001, i.e. 4x10^12 CFU, was collected between the 4th and 7th day of consumption of the fermented milk. The authors believed the survival rate in the faeces to be approximately 28.4%. According to the authors, the disparity between the survival rates measured in the faeces and the ileum may be explained by 3 hypothesis: i) methodological bias lead to an under-estimation of the survival rates in the ileum, ii) the possibility that the probiotic may have multiplied in the colon, iii) the fermented milk was consumed in different ways: a single intake (300 mL) for the ileum samples and three different intakes (100 mL each) during the studies on the faeces.

The two studies confirm that probiotics can travel through the digestive tract and reach the end of the small intestine alive. This is the case for L. casei 001 and L. bulgaricus. For S. thermophilus, it is harder to demonstrate this, given that the probiotic was only detected in the faeces of one individual out of 10. The authors do not rule out that prolonged consumption or a greater intake could have enabled it to be detected in more subjects. These two studies do not show however whether the probiotic rates detected are compatible with a biological effect in humans.

Modulation of the β-galactosidase activity of S. Thermophilus by the human gut flora

The publications discussed above treat the survival of probiotics in the human digestive tract. It is assumed that survival is a condition for their beneficial health effects produced by the probiotics. Is this so?

A team of French researchers from INRA (National Institute for Agronomic Research) has evaluated the functionality of the β-galactosidase in S. thermophilus DN-114 001 in vivo and in vitro, using appropriate biological tools, especially as relevant culture techniques are currently undergoing rapid evolution.

Previous results, published by the same team, had shown that S. thermophilus produced an active β-galactosidase during transit in germ-free mice (5, 6). In as far as, in this type of model, the only bacterial species present in the digestive tube of the mice was the S. thermophilus bacterium introduced for the study, it was not uncertain to measure the potential influence of the native flora on the expression of this enzyme. This is the target of this new publication. The authors have studied the initial kinetic, measured the survival rates of S. thermophilus and determined the activity of β-galactosidase in different compartments of the gut of mice that were either germ free or possessed human flora.

It should be remembered that the strain of S. thermophilus used in these studies had had its lactose operon genetically modified, introducing a gene coding for an enzyme called luciferase instead of the β-galactosidase gene. Luciferase expression can easily be highlighted by a light emitted by bacteria grown in a selective medium. This medium supports the growth of S. thermophilus from all 10 subjects enrolled in the study, although only 1 of these subjects was infected with S. thermophilus. The pink halos of streptococci from the yellow colonies and permits to clearly distinguish S. thermophilus from all other bacteria. Of course, the molecular tools have assets in terms of specificity of identification but it will be better if their use remains accompanied by traditional microbiology techniques, especially as relevant culture techniques are currently undergoing rapid evolution.

In the two murine models studied, S. thermophilus reached the caecum in 2 hours and its survival rate was close to 100%. In germ-free mice, β-galactosidase activity was detected in the second part of the small intestine and in the caecum/colon compartment. Although the enzyme is also activated in the second part of the small intestine of mice with human flora, it is inhibited drastically in the caecum/colon segment.

In the caecum/colon compartment, the human flora appears to interfere with the expression of the lactose operon whereas the integrity of S. thermophilus is not damaged and the bacteria stays alive. This observation implies that the resident flora is able to modify the physiology of S. thermophilus without affecting its viability.

These results show that S. thermophilus survives in the small intestine and that it expresses active β-galactosidase there. It should be stressed that it is in the small intestine that lactase activity is important since if the lactose is not digested by this part of the intestine it arrives in the colon where its fermentation provokes problems associated with its poor digestion. These results also support the idea that a dairy product containing a probiotic with β-galactosidase could be tolerated by people with a lactase deficiency. These studies in mice should be seen as preliminary to the results now awaited in humans.
Failure of *L. rhamnosus* to treat atopic dermatitis in newborns

Studies have suggested that probiotics can be beneficial in preventing and/or treating allergic disorders such as atopic dermatitis and allergy to milk proteins (7-11). In its turn, a research team from the Netherlands carried out a clinical study (randomized, double-blind placebo-controlled) in order to assess the effects of two milk formula products each containing a strain of the *Lactobacillus rhamnosus* probiotic on the symptoms of atopic dermatitis in newborns (12).

Fifty newborns (age < 5 months) suffering from atopic dermatitis were divided into 3 groups. One was given hydrolysed formula (control) and the other two were given the same formula supplemented either with a non-specified strain of *L. rhamnosus*, or with *L. rhamnosus* GG. The milk was consumed for 3 months and the probiotics were administered at a rate of 5x10⁹ CFU per 100 mL of formula.

The clinical development of the dermatitis was tested by the SCORAD test; allergic sensitivity was estimated by measuring IgG levels in the circulation and by the cutaneous response to a host of food allergens (e.g. milk proteins). Inflammation was estimated by measuring the number of eosinophils in the blood, X protein in the eosinophils in the urine, faecal α-antitrypsin and the production of IL-4, IL-5 and IFNγ cytokines by the lymphocytes after stimulation.

The results were clear: no significant statistical difference was measured between the groups whatever the clinical or biochemical parameters evaluated.

The probiotics used in this study had neither convincing effects on the immune system nor any significant clinical effects on atopic dermatitis. Consequently, the results clearly show that oral supplementation with one or the other of the two strains of *L. rhamnosus* tested has no significant impact on atopic dermatitis in newborns.

The results contradict a previous study (5) that showed that consumption of hydrolysed formula containing *L. rhamnosus* GG for one month caused an improvement of symptoms measured by SCORAD in newborns suffering from atopic dermatitis. However, in this study, the control group did not receive milk as the placebo but a lactoserum hydrolysate. Consequently, the potential effect of the probiotic could not be fully assessed with the protocol used.

In conclusion, the effects of probiotics on atopic dermatitis are today not established. To interpret the results of studies carried out in this area, care must be taken with the experiment protocol but also with the strain of the probiotic used, given that each bacterium strain has its own specific properties.

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Immune effect of probiotics in healthy humans - testing the dose-response relationship

Studies that assess the dose-response relationship of probiotics are rare. The one reported here assessed the immunomodulating capacity of *Bifidobacterium lactis* BB-12 and *Lactobacillus paracasei* ssp. *paracasei* CRL-431, administered at different doses to young adults (13). The clinical trial was conducted double-blind and placebo-controlled.

A group of 71 healthy young adults (aged 18-40) were given capsules containing a mixture (1/1) of two probiotics at doses of 0, 10⁸, 10⁹, 10¹⁰ and 10¹¹ CFU per day for 3 consecutive weeks.

Different parameters were measured in order to assess the immunomodulating capacity of the probiotics. Evaluation of phagocytosis was tested by measuring the number of eosinophils in the blood, X protein in the eosinophils in the urine, faecal α-antitrypsin and the production of IL-4, IL-5 and IFNγ cytokines by the lymphocytes after stimulation.

According to the authors, the mix of probiotics used in this study has no convincing effects on the immune system in healthy young adults; this was observed whatever the concentrations of probiotics used. The authors explain the absence of action that contradicts previous studies, by the fact that the majority of these studies were conducted using fermented milks containing probiotics and consequently the fermented milks per se may have an effect independent of the probiotics they carry. Furthermore, few studies have been carried out on healthy young adults, which unlike other group (e.g. the elderly or allergy-sufferers), are less sensitive to modulation of the immune system.

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probiotics treating cold and flu

The effects of probiotics would appear not to be restricted to the gastro-intestinal tract. A clinical study tested the benefits of long-term consumption of probiotics on the occurrence, duration and severity of colds and flu (14). 479 healthy adults were recruited and monitored from May 2001 or December 2001 until June 2002. Volunteers in the test group were asked to take one tablet daily, containing 5x10^7 CFU of the freeze-dried Bifidobacterium longum SP07/3, B. bifidum MF 20/5 and Lactobacillus gasseri PA 16/8 probiotic mixture and vitamins and minerals; those in the control group received the same tablet but only containing the vitamins and minerals. The two types of tablets were distributed double blind. The results of the trial were measured against clinical and immune system criteria. The clinical symptoms were assessed for the nose, pharynx and bronchi and the onset of headaches, myalgia, fever or conjunctivitis were noted (evaluation via a questionnaire). The cell immune response was assessed after quantifying sub-populations of T lymphocytes. In cases of infection, the viral agent provoking the infection was identified.

In comparison with the control, the occurrence of infections was not modified by taking probiotics. However, in subjects taking the probiotic, the average duration of an infectious attack was significantly reduced by two days (relative reduction of 21.5%, p<0.05) and the symptoms were less severe (p<0.05). After two weeks of probiotic consumption, a significant increase in the number of cytotoxic lymphocytes and suppressors (CD8+) was observed.

These results lead to the conclusion that the probiotic mix seems to have a positive effect on the severity and duration of viral infections of the respiratory tract in otherwise healthy adults. According to the authors, the impact on the parameters studied was slight, since it is probably masked by the immuno-stimulating action of the vitamins and minerals received by the control group.

New functionalities for probiotics

Conjugated linoleic acids (CLA) are a major focus of scientific research since numerous health benefits would appear to be linked to their consumption such as anti-carcinogenic and anti-atherogenic effects and the reduction of body fat mass. It is this latter ability that enabled a Korean research team to formulate the hypothesis that CLA-producing probiotics could help control weight (15). Their experiments were conducted on mice. The probiotic used was Lactobacillus rhamnosus PL60, a strain of human origin selected for its ability to produce CLA c9, t11 and t10, c12 in vitro. Three groups of mice were subjected to a fat-rich diet intended to fat them up. Two groups were given the probiotics and vitamins and minerals; those in the control group received the same tablet but only containing the vitamins and minerals. The two types of tablets were distributed double blind. The results of the trial were measured against clinical and immune system criteria. The clinical symptoms were assessed for the nose, pharynx and bronchi and the onset of headaches, myalgia, fever or conjunctivitis were noted (evaluation via a questionnaire). The cell immune response was assessed after quantifying sub-populations of T lymphocytes. In cases of infection, the viral agent provoking the infection was identified.

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Exploration of the anti-obesity effect

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Evaluation of the effects of probiotics on the human intestinal tract

The report on a project financed by the Food Standards Agency (FSA), a British government department responsible for food safety, has just been published. The project's goal was to supply the FSA with independent information on the effects of probiotics on the human intestine. The project was divided into three different areas, aiming to: compare the probiotics on sale in the United Kingdom, assess the survival of these probiotics in the gastro-intestinal medium and assess to what degree these probiotics can cause modifications to the composition of the gut flora. The results of this study are available online.

A probiotic reduces pulmonary lesions in mice

In mice infected with Streptococcus pneumoniae (pathogenic bacteria responsible for septicaemia, otitis and meningitis), consumption of Lactobacillus casei CRL431 led to a more rapid clearance of the pathogenic bacteria, a smaller number of pathogens in the lungs and a shorter period of septicaemia when compared with mice that had been given the probiotic. The probiotic caused activation of the phagocytes and production of pathogen-specific IgA and IgG. Consequently the probiotic generated an effective immune response at the same time as reducing the pulmonary lesions.

A probiotic for mothers that has no effect on newborn babies

The objective of this clinical trial was to determine if the post-natal administration of probiotics to mothers could have an effect on gastro-intestinal symptoms, crying and the development of the newborn’s gut microbiota. During their pregnancy and for the six months following childbirth, the women were given the Lactobacillus rhamnosus GG probiotic. There proved to be no difference between the newborns of these women and those of women who had taken a placebo. In this way, the administration of L. rhamnosus GG during the first months of life, while being well-tolerated by the babies, did not in any way affect the composition of their gut microbiota.

A bifidobacterium regulating the production of TGF-ß via the Smad7 protein

TGF-ß1 acts on the gut mucosa in numerous ways: tolerance, anti-inflammatory action, stimulation of IgA expression and the proliferation and differentiation of epithelial cells. The authors have shown that supplementing the feeding of premature babies with Bifidobacterium breve firstly increased the levels of TGF-ß1 in the blood and the expression of a TGF-ß (smad3) signal molecule and secondly reduced the expression of an antagonist signal molecule (smad7). These results show that the administration of B. breve to premature babies could attenuate inflammatory and allergic reactions by modulating TGF-ß signalling.

Role of commensal bacteria in intestinal immunity

Researchers have analyzed the immune responses caused in vitro in human gut epithelial cells by endogenic bacteria (Bifidobacterium infantis and Lactobacillus salivarius) and by a pathogenic bacterium (Salmonella typhimurium). S. typhimurium increases the expression of 36 of the 847 genes involved in immunity including NF-κB and IL-8 whereas neither of the commensal bacteria damage the expression of any of these genes. However, L. salivarius and B. infantis reduce the secretion of IL-8 and the pro-inflammatory responses generated by pathogenic bacteria, and stimulate production of IL-10 and TNF-α by the dendritic cells. It therefore appears that even if the gut epithelium remains quiescent in contact with L. salivarius and B. infantis, these bacteria exert an effect on the intestinal immune cells.

References:


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Yoghurts & fermented milks

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