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# Yoghurts & fermented milks

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## Lactobacillus bulgaricus - lessons from the genome sequence

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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.

van de Guchte M, Penaud S, Grimaldi C, Barbe V, Bryson K, Nicolas P, Robert C, Oztas S, Mangenot S, Couloux A, Loux V, Dervyn R, Bossy R, Bolotin A, Batto JM, Walunas T, Gibrat JF, Bessieres P, Weissenbach J, Ehrlich SD, Maguin E (2006). The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. Proc Natl Acad Sci USA. 13:9274-9279.  
Nicolas P, Bessières P, Ehrlich SD, Maguin E, van de Guchte M (2006). Extensive transfer of core genome genes between two *Lactobacillus* species found in the human gastrointestinal tract. Submitted manuscript.  
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Bolotin A, Quinquis B, Renault P, Sorokin A, Ehrlich SD, Kulakauskas S, Lapidus A, Goltsman E, Mazur M, Pusch GD, Fonstein M, Overbeek R, Kyprides N, Purnelle B, Prozzi D, Ngui K, Masuy D, Hancy F, Burteau S, Boutry M, Delcour J, Goffeau A, Hols P (2004). Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. Nat Biotechnol 22:1554-1558.

## 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 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 ( $5 \times 10^{10}$  CFU of *Streptococcus thermophilus* and  $6 \times 10^9$  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  $1,6 \times 10^9$  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  $10^8$  CFU.mL<sup>-1</sup> absorbed in a single intake).

In the faeces, the maximum quantity of *L. casei* DN-114 001, i.e.  $4 \times 10^7$  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* DN-114 001 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 consumption could have enabled it to be detected in more subjects. These two studies do not show however whether the probiotic rates collected are compatible with a biological effect in humans.

In an earlier study (3) the authors conclude that their results show that yoghurt bacteria do not survive transit. The microbiological approach that this study is based on was greatly different to the methods used in the two studies presented here: bacteria were identified directly by molecular tools without rigorous selection in a relevant medium of culture. Studies performed by Elli *et al.* and Oozer *et al.* tend to show that an appropriate selective approach using classic microbiology techniques can be used to evaluate survival adequately and that molecular techniques can be used in complementary fashion to confirm the results. All these results underline the influence of the methodological approach on results related to the assessment of the survival rates of 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 tools, especially as relevant culture techniques are currently available.

1• Elli M, Callegari ML, Ferrari S, Bessi E, Cattivelli D, Soldi S, Morelli L, Goupil Feuillerat N, Antoine JM (2006). Survival of yogurt bacteria in the human gut. *Appl Environ Microbiol* 72:5113-5117.

2• Oozer R, Leplingard A, Mater DDG, Mogenet A, Michelin R, Seksek I, Marteau P, Dore J, Bresson JL, Corthier G (2006). Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol* 72:5615-5617.

3• del Campo R, Bravo D, Canton R, Ruiz-Garbajosa P, Garcia-Albiach R, Montesi-Libois A, Yuste FJ, Abreira V, Baquero F (2005). Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers. *Appl Environ Microbiol* 71:547-549.

## Modulation of the $\beta$ -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 proof of functionality and, consequently, of the health effects produced by the probiotic. Is this so?

A team of French researchers from INRA (National Institute for Agronomic Research) has evaluated the functionality of the  $\beta$ -galactosidase in *Streptococcus thermophilus* *in situ* in mice (4).

Previous results, published by the same team, had shown that *S. thermophilus* produced an active  $\beta$ -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 until then possible 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 transit kinetics, measured the survival rates of *S. thermophilus* and determined the activity of  $\beta$ -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  $\beta$ -galactosidase gene. Luciferase expression can easily be highlighted due to the light emitted when the enzyme hydrolyses certain aldehydic substrates; the enzymatic activity can then be measured via luminometry. If the bacteria are alive, lactose activates the operon, triggering luciferase synthesis as if it were  $\beta$ -galactosidase. The experiment shows that major luciferase activity can be detected in the faeces of mice that had first been fed with a strain of modified *S. thermophilus* and consuming lactose-water.

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,  $\beta$ -galactosidase activity is 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 bacterium 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  $\beta$ -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  $\beta$ -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.

4• Mater DDG, Drouault-Holowacz S, Oozer R, Langella P, Anba J, Corthier G (2006). Beta-galactosidase production by *Streptococcus thermophilus* is higher in the small intestine than in the caecum of human-microbiota-associated mice after lactose supplementation. *Br J Nutr* 96:177-181. This work was supported by the Scientific Committee of SynDifrais.

5• Drouault S, Anba J, Corthier G. (2002). *Streptococcus thermophilus* is able to produce a beta-galactosidase active during its transit in the digestive tract of germ-free mice. *Appl Environ Microbiol* 68:938-941.

6• Cerf-Bensussan N. (2002). Un nouvel éclairage sur le rôle direct des bactéries lactiques vivantes dans la digestion du lactose. *Yaourts Laits Fermentés*, Lettre n°8, Editorial p.1.

## 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  $5 \times 10^9$  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  $\alpha$ -antitrypsin and the production of IL-4, IL-5 and IFN $\gamma$  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.

7• Majamaa H, Isolauri E. (1997). Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 99:179-185.

8• Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet*. 357:1076-1079.

9• Rautava S, Kalliomaki M, Isolauri E. (2002). Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol* 109:119-121.

10• Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A. (2003). Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. *J Allergy Clin Immunol* 111:389-395.

11• Weston S, Halbert A, Richmond P, Prescott SL. (2005). Effects of probiotics on atopic dermatitis: a randomised controlled trial. *Arch Dis Child* 90:892-897.

12• Brouwer ML, Wolt-Plompen SA, Dubois AE, van der Heide S, Jansen DF, Hoijer MA, Kauffman HF, Duiverman EJ. (2006). No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. *Clin Exp Allergy*. 36:899-906.

## 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,  $10^8$ ,  $10^9$ ,  $10^{10}$  and  $10^{11}$  CFU per day for 3 consecutive weeks.

Different parameters were measured in order to assess the immuno-modulating capacity of the probiotics. Evaluation of

phagocyte activity shows no statistically significant difference – whatever the probiotic dose – when comparing the results obtained from the subjects treated to those from the subjects given the placebo and those obtained before inclusion in the study. It was the same for the faecal IgA, for blood IgA, IgM and IgG (measured in the control group and among those receiving  $10^{10}$  CFU), for the production of cytokines (IL-10 and IFN $\gamma$ ) by lymphocytes stimulated *in vitro*.

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.

13• Christensen HR, Larsen CN, Kaestel P, Rosholm LB, Sternberg C, Michaelsen KF, Frokiaer H. (2006). Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. *FEMS Immunol Med Microbiol* 47:380-390.

This scientific letter “Yoghurts & fermented milks” is also available on the following website:

[www.maison-du-lait.com](http://www.maison-du-lait.com)

## 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  $5 \times 10^7$  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 cri-

teria. 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.

14• de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J. (2006). Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. *Vaccine*. 24:6670-6674.

## Exploration of the anti-obesity effect

### of a probiotic producing conjugated linoleic acid

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 fatten them up. Two groups were given the probiotic in addition in quantities of  $1 \times 10^7$  or  $1 \times 10^9$  CFU/day for 8 weeks, the third group was the control group (diet containing no probiotics). A fourth group of mice was given standard diet (second control group).

The lipid-rich diet clearly caused a significant change in body mass over the 8 weeks. The differences between the groups were measurable from week 3 and significant from week 5. The control

mice subjected to the lipid diet were clearly obese from week 5 and their weight was significantly higher than that of the mice who were given the probiotics. In mice subjected to the lipid diet, an accumulation of adipose tissue was observed in the epididymis, the mesentery and around the kidneys.

The lipid diet caused an increase in total cholesterol, LDL and HDL in the mice in the three groups compared to the mice given the standard diet. The serum leptin levels increased in all the mice receiving the lipid diet but significantly less in those given the probiotics.

The consumption of probiotics caused an increase of ARNm levels of the UCP-2\* protein in the epididymis that was almost double that of the two controls; but the probiotic did not affect the ARNm levels of the PPAR\*\* receptors.

The results of this study show that the oral consumption of a probiotic that produces linoleic acid can control weight gain caused by a lipid-rich diet. It in fact provides an anti-obesity effect that would appear to be based, at least partially, on the modulation by the probio-

tic of the production of the UCP-2 protein in the adipose tissue.

This study is the first to explore the role of probiotic in weight management. However, another study (16) shows that fat loss via the CLA can lead in mice to a lipodystrophia associated with severe side effects (hyperinsulinemia, insulin-resistance, hepatic steatosis). Consequently, it isn't time to make extrapolations to humans.

\*The UCP-2 (uncoupling protein 2) gene codes for a protein that would appear to burn up a part of the excess calories and dissipate them as heat. This gene exists in both mice and humans.

\*\*PPAR (peroxisome proliferator activated receptors) are nuclear membrane receptors of the cells activated by linking with certain fatty acids or lipids involved in lipogenesis.

15• Lee HY, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH. (2006). Human originated bacteria, *Lactobacillus rhamnosus* PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim Biophys Acta*. 1761:736-744.

16• Poirier H, Niot I, Clement L, Guerre-Millo M, Besnard P. (2005). Development of conjugated linoleic acid (CLA)-mediated lipotrophic syndrome in the mouse. *Biochimie*. 87:73-79.

## 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.

17• Gibson GR, Rouzaud G, Brostoff J, Rayment N (2006). An evaluation of probiotic effects in the human gut: microbial aspects. Final Technical report for FSA project ref. G01022. <http://www.food.gov.uk/multimedia/pdfs/probioticreport.pdf>

## 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.

18• Racedo S, Villena J, Medina M, Aguero G, Rodriguez V, Alvarez S. (2006). *Lactobacillus casei* administration reduces lung injuries in a *Streptococcus pneumoniae* infection in mice. *Microbes Infect* 8:2359-2366.

## 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 microflora. 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 microflora.

19• Rinne M, Kalliomaki M, Salminen S, Isolauri E. (2006). Probiotic intervention in the first months of life: short-term effects on gastrointestinal symptoms and long-term effects on gut microbiota. *J Pediatr Gastroenterol Nutr* 43:200-205.

## A bifidobacterium regulating the production of TGF-β via the Smad7 protein

TGF-β1 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-β1 in the blood and the expression of a TGF-β (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-β signalling.

20• Fujii T, Ohtsuka Y, Lee T, Kudo T, Shoji H, Sato H, Nagata S, Shimizu T, Yamashiro Y. (2006). *Bifidobacterium breve* enhances transforming growth factor beta1 signaling by regulating Smad7 expression in preterm infants. *J Pediatr Gastroenterol Nutr* 43:83-88.

## 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.

21• O'Hara AM, O'Regan P, Fanning A, O'Mahony C, Macsharry J, Lyons A, Bienenstock J, O'Mahony L, Shanahan F. (2006). Functional modulation of human intestinal epithelial cell responses by *Bifidobacterium infantis* and *Lactobacillus salivarius*. *Immunology*. 118:202-215.

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