

IN THIS ISSUE

- Probiotics preventing *intestinal infections*
- Probiotics protecting the intestinal mucosa : *hypothesis for a mode of action*
- Probiotics modulating the immune response : *potential implications for treating IBD*
- Probiotics and prophylaxis of *intestinal inflammations*
- Improvement in lactose digestion with *Kefir fermented milk*
- Using lactobacillus *to prevent atopic disease*
- The DNA of yoghurt starter as the carrier *of an immunostimulating oligonucleotide*

This survey letter on lactic acid bacteria is produced by the scientific committee of

SYNDIFRAIS

Publishing director

Arnauld de Miollis (Syndifrais)

Scientific coordinator

Brigitte Laurent

Reviewing board,

the Scientific Committee of Syndifrais

Jean Louis Bresson (Hôpital des enfants

malades, Paris), Nadine Cerf-Bensussan

(Hôpital Necker, Paris), Jean Fioramonti

(INRA, Toulouse), Robert Ducluzeau

(Directeur de recherche INRA),

Jean-Marie Eustache (MLC),

Irene Lenoir-Wijnkoop (Danone Vitapole),

Marie-France Pagerey (Affaires scientifiques

et réglementaires, Nestlé France),

Patricia Ramos (Yoplait)

Scientific Survey

Judith Benrekassa (Cerin)

Editor



Responsible for the information

Eric Labouze (BIO Intelligence Service)

Writer

Lamya Moulay (BIO Intelligence Service)

Yoghurts & fermented milks

November 2003 - Letter N° 15

Health • Nutrition • Flora

SCIENTIFIC SURVEY . LACTIC ACID BACTERIA . PROBIOTICS

Must probiotics be of human origin to be effective on humans ?

Robert Ducluzeau

Research into new probiotics that can be included in human foodstuffs is continuing actively. Most of the authors who describe new probiotics start from the premise that the bacterial strains used must come from the flora of the human digestive tract or believe this to be so obvious that they do not even need to state it. But what unquestionable scientific arguments exist to affirm that probiotics for human consumption must necessarily be of human origin ?

For many authors, a strain that is of human origin implants itself more easily in the digestive tract, stays there longer and is better tolerated. However, the fact that a particular strain originates in the digestive tract does not necessarily mean that it is able to implant itself there, i.e. to multiply and remain in the digestive tract of the person who ingests it, especially given the multiple subcultures it has been subjected to before being used as a probiotic. It has been now widely shown that none of the probiotics on the market implant themselves in the digestive tract and as we already stated in Letter number 5, it is better that way! On the other hand, attachment to the wall of the digestive tract, often seen to be a condition for a strain's implantation, is host-dependent, since only strains of human origin are capable of developing systems for attaching themselves to human mucus membranes. But nothing proves that this characteristic is indeed not more harmful than it is useful for the host (Letter 7) and it has certainly not been shown for any of the strains currently used as probiotics that they can attach themselves to the mucosa. Furthermore, "tolerance", in the immunological sense of the word, is in no way the prerogative of bacteria of the digestive tract flora. A strain of these indigenous bacteria, carried into the blood of its host, causes an immune reaction. We now know that, to be effective, a probiotic must stay alive during its passage through the digestive tract. But it has been shown that resistance to the hostile conditions encountered in the intestine is linked rather to the strain itself than to its ecological origins.

Another argument put forward to justify choosing strains from the human digestive tract concerns the major problem of the harmlessness of probiotic strains. Certainly the widespread choice of lactic bacteria as probiotics arises from their longstanding use in human foodstuffs without any harmful activity being observed. However, a few strains of lactic bacteria do exist that can cause serious infection, in particular in immunodepressed patients. It has been observed that these strains belong, for the most part, to species whose home is precisely the human digestive tract, such as *Enterococcus faecalis*. Indeed, very often, such infections are linked to bacteria in the individuals' own flora and not to ingested bacteria. All in all, the fact a particular strain of lactic bacteria is of human origin is in no way an assurance that it is totally harmless.

Finally, no patent scientific facts exist that corroborate the need for probiotics to be of human origin. Indeed, the probiotics most widely consumed around the world, lactic bacteria in yoghurt, are not of human origin. However, their effectiveness *in vivo*, at least as far as their lactase activity is concerned, is now definitively proven.

Ducluzeau R. (2002). Le concept de probiotiques : historique, définition et principales caractéristiques. Antibiotiques 4, 234-238.

Collins J.K., Thornton G., Sullivan G.O. (1998). Selection of probiotic strains for human applications. Int. Dairy Journal 8, 487-490.

Probiotics preventing intestinal infections

The intestinal epithelium spearheads a number of functions ensuring intestinal homeostasis. It acts as a barrier, preventing germs and macromolecules present in the intestinal lumen from entering the intestinal chorion, and plays a role in regulating the transport of fluids and electrolytes. Alterations of the barrier and transport functions are the first consequences of digestive disorders resulting in particular in the symptoms of diarrhoea. It has been shown *in vitro* that the infection of the intestinal cells by invasive bacteria upsets both transport and the barrier effect (1). The hypothesis formulated by Resta-Lenert & Barrett is that the pre-treatment of cultured epithelial cells with certain probiotics could protect them from the harmful effects resulting from infection by a pathogenic bacterium (2).

The researchers chose to analyse, *in vitro*, the effects of the probiotics *L. acidophilus* and *S. streptococcus* on human epithelial cells (HT29 cells) that may have been infected by the *E. coli* bacterium, entero-invasive EIEC (entero-invasive *E. coli*).

Together or separately, *in vitro* these probiotics were able to interact with intestinal epithelial cells and EIEC and limit EIEC colonization of the cells. In fact, the number of adherent EIEC bacteria is reduced when the epithelial cells are pre-incubated with one or other of the probiotics (ratio 1/1). EIEC adhesion is totally stopped when the number of probiotics rises, while the number of EIEC bacteria remains constant. Simultaneously adding EIEC and the probiotics inhibits the adhesion of EIEC to the epithelial cells.

One of the major effects of intestinal pathogens and EIEC is to reduce trans-epithelial resistance* (TER) in the intestine (3). The authors studied the ability of probiotics to abrogate this harmful effect. It appears that if intestinal cells come into contact with probiotics before EIEC is added or if EIEC and pro-

biotics are added simultaneously to the intestinal cells, then the decrease of TER that EIEC normally causes, is reduced.

Since modification of TER is synonymous with modifications to permeability, the authors sought to measure the flow of two probe molecules through a layer of epithelial cells, infected or not by EIEC. After infection with EIEC, the permeability of the intestinal cells increases and this increase is proportionally greater the smaller the probe molecule. On the other hand, if the intestinal cells are incubated with probiotics before infection, this prevents the changes in permeability caused by EIEC. This is however prevented if the probiotics are previously inactivated by antibiotic treatment or heat. Furthermore, the authors have also been able to show that these probiotics are able to prevent increases in the secretion of electrolytes (chloride ions) caused by EIEC.

Alongside changes to TER, there will also be negative modifications to the cytoskeleton and the tight junctions. An analysis of the state of phosphorylation of some proteins of the cytoskeleton and the tight junctions shows that pre-incubation of intestinal cells with probiotics prevents the changes brought about by EIEC to the proteins and tight junctions (such as a reduction in the phosphorylation of the serine residue of occludin and the tyrosine residue of ZO-1).

Given the critical role played by EGF** in gastro-intestinal physiology and repair to the epithelia (4), the authors put forward the hypothesis that the beneficial effect of probiotics may concern restored signalling of EGF receptors in epithelial cells infected with EIEC. Indeed, studies had showed that EIEC infection inhibited and/or increased a degradation in EGF receptors (3, 5). When epithelial cells are placed in contact with probiotics and are infected with EIEC and then stimulated with EGF, activation of the EGF receptors significantly

remains in comparison to cells infected in the absence of probiotics. However, this effect is not observed when probiotics are added to the cell cultures at the same time as EIEC or when the probiotics have been inactivated by antibiotics.

These results, obtained *in vitro*, suggest that certain probiotics are able to interfere with enteric pathogens via various mechanisms, on the one hand preventing the adhesion of the pathogen to the epithelium and on the other maintaining the integrity of the barrier and transport functions exerted by the intestinal epithelium. Moreover, the probiotics studied lose these positive effects when they are previously inactivated with an antibiotic treatment or heat.

* Trans-epithelial resistance (TER) is the measurement of the electrical resistance of a layer of cells in culture. It is expressed in Ohm/cm².

** EGF (epidermal growth factor) is a growth factor whose role is to stimulate the growth, proliferation and differentiation of the cells of the epidermis. It acts on a membrane tyrosine kinase receptor and has nuclear effects. EGF regulates the barrier function, transport, epithelial growth and differentiation..

1• Resta-Lenert S, Barrett KE (2002). Enteroinvasive bacteria alter barrier and transport properties of human intestinal epithelium: role of iNOS and COX-2. *Gastroenterology*. 122(4):1070-87.

2• Resta-Lenert S, Barrett KE (2003). Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive *Escherichia coli* (EIEC). *Gut*. 52(7):988-97.

3• Resta-Lenert S, Barrett KE (2001). Modulation of tight junction structure by enteroinvasive *E. coli* (EIEC) is prevented by H₂O₂ scavengers and protein kinase (PKC) inhibitors. *Gastroenterol* 120:3802.

4• Riegler M, Sedivy R, Sogukoglu T, Cosentini E, Bischof G, Teleky B, Feil W, Schiessel R, Hamilton G, Wenzl E (1997). Effect of growth factors on epithelial restitution of human colonic mucosa *in vitro*. *Scand J Gastroenterol*. 32(9):925-32.

5• Resta-Lenert S, Barrett KE (2000). Increased expression of iNOS and COX-2 is associated with activation of chloride currents in HT29/Cl.19A cells infected by enteroinvasive bacteria. *Gastroenterol*. 118:4325.

This scientific letter "Yoghurts & fermented milks" is also available on the following websites:

www.maison-du-lait.com and www.syndifrais.org

Probiotics protecting the intestinal mucosa :

hypothesis for a mode of action

One of the strategies deployed by the intestinal mucosa to protect itself from colonisation by pathogens is to inhibit their adhesion to the epithelial surfaces. One of the mechanisms it uses is the production of mucins. These glycoproteins, secreted by the epithelial cells, limit access to the mucous membrane by creating steric crowding and therefore building a physical barrier to protect against pathogens (6).

Earlier works (7) showed that the probiotics *L. plantarum* 299v and *Lactobacillus* GG were capable of inhibiting the adhesion of some enteropathogenic strains of *E. coli* (EPEC strain) to human epithelial cells (HT29 line) cultivated *in vitro* and also that these probiotics were capable of stimulating the expression of RNA, specific messengers of the extracellular mucins (MUC3) in the HT29 cells.

The questions posed by Mack *et al* (8) aim to define whether: 1) adhesion of the probiotic to the mucous membrane is necessary in order to provoke mucin

secretion 2) if mucin production is biologically significant.

Adhesion tests show that *Lactobacillus* GG adheres 10 times more than *L. plantarum* 299v that itself adheres 10 times more than *L. acidophilus* DDH. Production of MUC3 is significantly greater when the HT29 cells are incubated with *Lactobacillus* GG or with *L. plantarum* 299v. *L. acidophilus* DDH does not seem to influence this production. Furthermore, a variant of the strain *L. plantarum* 299v that is incapable of adhering to the intestinal cells is also incapable of stimulating MUC3 expression. This data suggests that probiotics can stimulate MUC3 expression on the condition that they can adhere to the intestinal cells.

Adhesion inhibition tests show that simultaneous incubation of HT29 and EPEC in the presence of *Lactobacillus* GG or *L. plantarum* 299v prevents the adhesion of EPEC to the HT29 cells. However, variants of strains of *L. plantarum* 299v and *L. acidophilus* DDH were not able,

under the same conditions, to prevent the adhesion of EPEC to HT29 cells.

These results confirm that certain probiotics are able to stimulate the intestinal epithelial cells to produce mucins. This ability would appear to be active when the probiotics adhere to the epithelial cells. Finally, an increase in the secretion of extracellular mucins could play a role in inhibiting the adhesion of pathogenic bacteria to the intestinal epithelium. In other words, these probiotics would appear to act as protectors, by reinforcing the physical barrier effect exerted by the intestinal mucosa.

6• Dai D, Nanthkumar NN, Newburg DS, Walker WA (2000). Role of oligosaccharides and glycoconjugates in intestinal host defense. *J Pediatr Gastroenterol Nutr.* 30 Suppl 2:S23-33.

7• Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA (1999). Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. *Am J Physiol.* 276:G941-50.

8• Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA (2003). Extracellular MUC3 mucin secretion follows adherence of *Lactobacillus* strains to intestinal epithelial cells *in vitro*. *Gut.* 52(6):827-33.

Probiotics modulating the immune response :

potential implications for treating IBD

Relatively little is known about the possible interactions between bacteria and the host's intestinal mucosa. The majority of bacteria constituting the intestinal flora coexist without any harmful effects and may even play a positive role in the host's health. Currently several studies suggest that a symbiotic relationship exists between these two entities.

The team of Malagelada *et al*, that is studying the involvement of bacteria in inflammatory bowel diseases (IBD), has looked particularly at the interaction between non-pathogenic bacteria and the host's immuno-inflammatory mechanisms (9). These researchers have chosen to analyse the effects of different strains of probiotics on the spontaneous production of cytokines by colic explants coming either from healthy individuals or individuals suffering from Crohn's disease. This type of model has the advantage of allowing a study of host-bacteria interactions using both the epithelial interfaces and the immuno-competent cells in their natural surroundings.

These experiments, conducted *ex vivo*, show that three strains of *L. casei* DN-114 001, DN-114 056 and ATCC 334 are

able to reduce the spontaneous production of TNF α by normal colic explants. Strain DN-114 001 is also able to reduce production of IL8 and IL10 (cf. table below). On the contrary, the non-pathogenic strain *E. coli* stimulates production of TNF α and IL10.

Production of cytokines by normal colic explants in contact with non-pathogenic bacteria (in comparison with explants cultivated without bacteria)

	TNF α	IL8	IL10
<i>L. casei</i> DN-114 001	↓	↓	↓
<i>L. casei</i> DN-114 056	↓	ns	↓
<i>L. casei</i> ATCC 334	↓	ns	ns
<i>L. bulgaricus</i> LB10	ns	no effect	ns
<i>E. coli</i>	↑	no effect	↑

ns : not significant

Cells from explants taken from patients suffering from Crohn's disease produce more TNF α than normal cells. However, when these cells are incubated with *L. casei* DN-114 001, the production of TNF α is reduced significantly. The co-culture of these cells with *L. casei* DN-114 001 and *E. coli* causes a significant reduction in the production of TNF α

whereas the co-culture of these cells with *E. coli* alone stimulates production of this cytokine.

These results show that non-pathogenic bacteria can influence the production of cytokines in the cells of the colic explants, but with differing profiles: the three strains of *L. casei* moderate the inflammatory response (by moderating the spontaneous production of cytokines) whereas the strain of *E. coli*, even though it is non-pathogenic, exacerbates it. Furthermore, strain *L. casei* DN-114 001 can block the production of TNF α stimulated by the presence of *E. coli*.

The results support recent suggestions that probiotics can be used to modulate the immune response in the human intestine, in particular in patients suffering from inflammatory bowel diseases.

* IBD (inflammatory bowel diseases) includes Crohn's disease and ulcerative colitis.

9• Borruel N, Casellas F, Antolin M, Llopis M, Carol M, Espiñ E, Naval J, Guarner F, Malagelada JR (2003). Effects of nonpathogenic bacteria on cytokine secretion by human intestinal mucosa. *Am J Gastroenterol.* 98(4):865-70.

Probiotics and prophylaxis of intestinal inflammations

Promising results have been obtained with probiotics for treating colitis in mice and as a preventive treatment against relapses of ulcerative colitis in humans (10, 11, 12). In particular, the efficacy of a probiotic preparation VSL#3* has been shown to be an adjuvant treatment for chronic pouchitis** (13) and ulcerative colitis (14) and in the prevention of post-operative flare-up of Crohn's disease (15).

Gionchetti *et al* (16) used VSL#3 with the aim of comparing the efficacy of this preparation with that of a placebo in the prevention of pouchitis in the year following the operation. The clinical trial was double-blind and placebo-controlled. The 40 patients, who had all undergone ileal-pouch anal anastomosis (IPAA) for ulcerative colitis, were asked to consume, just after the operation and for a duration of one year, one capsule per day of VSL#3 or a placebo. Every three months, the patients underwent clinical, histological and endoscopic examinations.

At the end of the one-year study, 18 of the 20 patients having received probio-

tics no longer suffered episodes of acute pouchitis as opposed to 12 of the 20 who had received the placebo. The significant difference ($P < 0.05$) in the onset of pouchitis between the two patient-groups shows that treatment with VSL#3 is relatively effective in preventing this type of inflammation. The results support the current hypothesis according to which probiotics appear to have a positive effect on inflammatory intestinal diseases, at least when they are consumed in large quantities and long-term.

* VSL#3 is a mixture of 8 species of probiotics - *L. casei*, *L. plantarum*, *L. acidophilus*, *L. bulgaricus*, *B. longum*, *B. breve*, *B. infantis* and *S. thermophilus*. Each VSL#3 capsule contained 9×10^{11} freeze-dried bacteria.

** Pouchitis is an inflammation (of unknown etiology) of a pouch inserted between the anus and the small intestine to replace the rectum. The operation is performed in cases of ulcerative colitis.

10• Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M (1997). Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther.* 11(5):853-8.

11• Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT (1999). Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet.* 354(9179):635-9.

12• Kruis W, Fric P, Stolte M (2001). The Mutaflor study group. Maintenance of remission in ulcerative colitis is equally effective with *Escherichia coli* Nissle 1917 and with standard mesalazine. *Gastroenterol.* 120:A680 (abstr).

13• Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M (2000). Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterol.* 119(2):305-9.

14• Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M (1999). Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther.* 13(8):1103-8.

15• Campieri M, Rizzello F, Venturi A, Poggioli G, Ugolini F, Helwig U, Amadini C, Romboli E, Gionchetti P (2000). Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn's disease a randomised controlled study vs mesalazine. *Gastroenterol* 118:A4179 (abstr).

16• Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M (2003). Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterol.* 124(5):1202-9.

Improvement in lactose digestion with Kefir fermented milk

Lactose maldigestion is the inability to fully digest lactose, the main carbohydrate in milk. This inability is the result of a lactase deficiency. Lactase is an enzyme normally found in the small intestine. Individuals unable to digest lactose usually avoid consumption of dairy products, thus depriving themselves of an important nutritional source of calcium. However, it is generally accepted that consuming fresh fermented dairy products, in particular yoghurt, improves lactose digestion (17, 18). Studies carried out on mice indicate that this effect is linked to the presence of β -galactosidase supplied by the lactic bacteria present in fermented milk (19).

An American team tested whether Kefir*, a fermented milk on sale in the USA, was able to improve lactose digestion (20). Forty adults, whose inability to digest lactose was assessed by the breath hydrogen test**, were divided into 5 groups, each consuming one of the following products: Milk, natural Kefir, flavoured Kefir, natural yoghurt or flavoured yoghurt.

The quantities of hydrogen exhaled by those individuals who had consumed natural and flavoured yoghurt and natural Kefir are small and statistically equivalent. Consuming milk results in a greater quan-

	Breath hydrogen level (ppm x hours)	β -galactosidase activity (units)
Milk	224 \pm 39	0
Natural Kefir	87 \pm 37	5.4
Flavoured Kefir	156 \pm 26	5.2
Natural yoghurt	76 \pm 14	3.4
Flavoured yoghurt	98 \pm 17	3.2

β -galactosidase activity is measured by spectrophotometry with ONPG as the substrate.

tity of breath hydrogen than consuming natural yoghurt ($P < 0.001$), natural Kefir ($P < 0.005$) and flavoured yoghurt ($P = 0.031$). The results obtained with milk and flavoured Kefir are not significantly different.

From the criteria of both breath hydrogen and clinical symptoms (flatulence and abdominal pain), this study shows that consuming fermented milk improves lactose digestion and that natural Kefir is as effective as natural yoghurt.

The differences highlighted between the natural and flavoured products can be explained, according to the authors, by the fact that the flavoured products

contain added carbohydrates (fruit fructose, fruit syrups) that appear to monopolise β -galactosidase activity to their own advantage and to the detriment of lactose.

* Kefir is milk fermented with different strains of lactic bacteria and yeasts.

** Where the intestinal mucosa prevents lactose from being properly digested, the ingested lactose is not hydrolysed and therefore not absorbed by the small intestine. It therefore goes into the colon, where it is fermented and results in the production of organic acids and gas, including hydrogen. The hydrogen is reabsorbed and eliminated via the lungs. Therefore, malabsorption of lactose is characterised by an increased concentration of breath hydrogen of at least 20 ppm above the basal level (Cochet *et al* 1981).

17• Pelletier X, Laure-Boussuge S, Donazzolo Y (2001). Hydrogen excretion upon ingestion of dairy products in lactose-intolerant male subjects: importance of the live flora. *Eur J Clin Nutr.* 55(6):509-12.

18• Kolars JC, Levitt MD, Aouji M, Savaiano DA (1984). Yoghurt--an autodigesting source of lactose. *N Engl J Med.* 5;310(1):1-3.

19• Drouault S, Anba J, Corthier G (2002). *Streptococcus thermophilus* is able to produce a β -galactosidase active during its transit in the digestive tract of germ-free mice. *Appl Environ Microbiol.* 68(2):938-41.

20• Hertzler SR, Clancy SM (2003). Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *J Am Diet Assoc.* 103(5):582-7.

Using lactobacillus to prevent atopic disease

The first convincing work to examine the effect of consuming probiotics on the prevention of atopic disease have been published in the Lancet by a Finnish team led by Isolauri (21). This was a randomised study, carried out double-blind and placebo-controlled in which *Lactobacillus* GG was prescribed to pregnant women suffering from allergies (atopic eczema, allergic rhinitis or asthma) and to their newborn babies from birth to the age of 6 months. By the age of 2, the frequency with which the children who had received the probiotic suffered from atopic eczema was half that of those children who had received the placebo.

These encouraging results did not however answer the question of children of more than two years' old. The same team then studied whether the preventive effect of *Lactobacillus* GG on atopic diseases was extended beyond the early years. To this end they re-examined the previously studied population (22). At the age of four, atopic eczema was diagnosed in 14 of the 53 infants who had

consumed the probiotic, against 25 of the 54 who had received the placebo (relative risk 0.57, 95 % CI 0.33-0.97).

A Danish team conducted a similar study using other strains of *Lactobacillus* (23). In this study, which was also randomised, double-blind and placebo-controlled, the probiotics *Lactobacillus rhamnosus* 19070-2 and *Lactobacillus reuteri* DSM 122460 were given together to children aged between 1 and 13 years over a six-month period. The association of the two probiotics was effective, at all ages, in managing atopic dermatitis, in so far as 56 % of the patients who consumed the probiotics saw an improvement in their eczema (measured on the SCORAD index) against only 15 % of those who were given the placebo.

Therefore, the consumption of lactobacilli seems to be effective in reducing the onset and magnitude of outbreaks of atopic dermatitis in children. According to the hypothesis of the Finnish authors, early colonisation of the intestine is crucial in ensuring good maturation in the

infant immune system. Administration of probiotics could provide enough stimulus for this maturation. This hypothesis supports the theory put forward by hygienists according to which excessive hygiene reduces early exposure to microbes and leads to an increased risk of allergy (24).

21• 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(9262):1076-9.

22• Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E (2003). Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. *Lancet*. 361(9372):1869-71.

23• 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(2):389-95.

24• Kalliomaki M, Isolauri E (2003). Role of intestinal flora in the development of allergy. *Curr Opin Allergy Clin Immunol*. 3(1):15-20.

The DNA of a yoghurt starter as the carrier of an immunostimulating oligonucleotide

The immuno-modulating effects of yoghurt are today widely documented. Research is currently underway to identify the immune parameters modulated by the consumption of lactic ferments and to identify bacterial factors able to interact with the host's immune system (25).

It has recently been shown that bacterial DNA, via CpG palindromic sequences (26) and the DNA of *L. gasseri* via specific nucleotidic patterns (27), were able to cause proliferation of B cells *in vitro*. A Japanese team of researchers has analysed the DNA of *L. bulgaricus* and *S. thermophilus* yoghurt starters with the objective of identifying an immunostimulating DNA sequence (28).

Chromosomal DNA was purified from 9 isolates of *L. bulgaricus* and 6 isolates of *S. thermophilus*. Its ability to stimulate proliferation was then tested on B cells obtained from spleen and Peyer's patches of mice (these cells were cultivated for 48 hours in the presence of DNA

and proliferation was assessed via radio-labelled uridine).

All the DNA stimulated, to various degrees, the proliferation of B lymphocytes but only that isolated from strains of *L. bulgaricus* NIAI B6 and *S. thermophilus* OLS3002 was able to cause a significant proliferation of the Peyer's patch cells. Since it caused significant proliferation of both cell types, the strain *L. bulgaricus* NIAI B6 was used to identify the nucleotidic pattern responsible for this stimulating activity.

The mitotic activity of DNA towards B cells was significantly increased. Ten homologous sequences of nucleotides were identified as potential mitogens. This included one CpG-like (5'-CGGCACGCTCACGATTCTTG-3') pattern as an immuno-stimulatory oligonucleotide but it did not contain the specific CpG palindromic structure for mitogenic activity of B cells. These homologous sequences were linked to B cells and sti-

mulated the proliferation of CD69+ cells in Peyer's patches.

This study shows that *L. bulgaricus* NIAI B6 carries a genomic pattern able to stimulate the proliferation of certain immune cells *in vitro*. It can therefore be expected that this probiotic is able to modulate local immunity *in vivo* via the stimulation of lymphoid tissues within the intestinal mucosa.

25• Meydani SN, Ha WK (2000). Immunologic effects of yogurt. *Am J Clin Nutr*. 71(4):861-72.

26• Krieg AM, Yi AK, Matson S, Waldschmidt TJ, Bishop GA, Teasdale R, Koretzky GA, Klinman DM (1995). CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature*. 374(6522):546-9.

27• Kitazawa H, Ueha S, Itoh S, Watanabe H, Konno K, Kawai Y, Saito T, Itoh T, Yamaguchi T (2001). AT oligonucleotides inducing B lymphocyte activation exist in probiotic *Lactobacillus gasseri*. *Int J Food Microbiol*. 65(3):149-62.

28• Kitazawa H, Watanabe H, Shimosato T, Kawai Y, Itoh T, Saito T (2003). Immunostimulatory oligonucleotide, CpG-like motif exists in *Lactobacillus delbrueckii* ssp. *bulgaricus* NIAI B6. *Int J Food Microbiol*. 85(1-2):11-21

LAB-DOC bibliographic selection

The data base LAB-DOC organised by SYNDIFRAIS, brought together the bibliographic references of the international scientific publications accompanied by the authors' summaries.

29• Akyol S, Mas MR, Comert B, Ateskan U, Yasar M, Aydogan H, Deveci S, Akay C, Mas N, Yener N, Kocar IH (2003).

The effect of antibiotic and probiotic combination therapy on secondary pancreatic infections and oxidative stress parameters in experimental acute necrotizing pancreatitis. *Pancreas*. 26(4):363-7.

30• Drisko JA, Giles CK, Bischoff BJ (2003).

Probiotics in health maintenance and disease prevention. *Altern Med Rev*. 8(2):143-55.

40• Fuglsang A, Rattray FP, Nilsson D, Nyborg NC (2003).

Lactic acid bacteria: inhibition of angiotensin converting enzyme in vitro and in vivo. *Antonie Van Leeuwenhoek*. 83(1):27-34.

41• Hori T, Kiyoshima J, Yasui H (2003).

Effect of an oral administration of *Lactobacillus casei* strain Shirota on the natural killer activity of blood mononuclear cells in aged mice. *Biosci Biotechnol Biochem*. 67(2):420-2.

42• Kos B, Suskovic J, Vukovic S, Simpraga M, Frece J, Matosic S (2003).

Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *J Appl Microbiol*. 94(6):981-7.

43• Laake KO, Line PD, Aabakken L, Lotveit T, Bakka A, Eide J, Roseth A, Grzyb K, Bjorneklett A, Vatn MH (2003).

Assessment of mucosal inflammation and circulation in response to probiotics in patients operated with ileal pouch anal anastomosis for ulcerative colitis. *Scand J Gastroenterol*. 38(4):409-14.

44• Maassen CB, Boersma WJ, van Holten-Neelen C, Claassen E, Laman JD (2003).

Growth phase of orally administered *Lactobacillus* strains differentially affects IgG1/IgG2a ratio for soluble antigens: implications for vaccine development. *Vaccine*. 21(21-22):2751-7.

45• McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, Fitzgibbon J, O'Sullivan GC, Kiely B, Collins JK, Shanahan F (2003).

Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. *Gut*. 52(7):975-80.

46• Sanders ME (2003).

Probiotics: considerations for human health. *Nutr Rev*. 61(3):91-9.

47• Schultz M, Linde HJ, Lehn N, Zimmermann K, Grossmann J, Falk W, Scholmerich J (2003).

Immunomodulatory consequences of oral administration of *Lactobacillus rhamnosus* strain GG in healthy volunteers. *J Dairy Res*. 70(2):165-73.

48• Shanahan F (2003).

Probiotics: a perspective on problems and pitfalls. *Scand J Gastroenterol Suppl*. (237):34-6.

49• Sybesma W, Starrenburg M, Kleerebezem M, Mierau I, de Vos WM, Hugenholtz J (2003).

Increased production of folate by metabolic engineering of *Lactococcus lactis*. *Appl Environ Microbiol*. 69(6):3069-76

Your suggestions and comments will draw all our attention. Please send them to :

SYNDIFRAIS

42 rue de Châteaudun • 75314 Paris Cedex 9
Phone : 33 1 49 70 72 30 • Fax : 33 1 42 80 63 90
e.mail : syndifrais@syndifrais-syndilait.org