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

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# Yoghurts & fermented milks

March 2004 - Letter N° 17

Health • Nutrition • Flora

SCIENTIFIC SURVEY . LACTIC ACID BACTERIA . PROBIOTICS

## edito

### Effects of probiotics on the digestive tube - the major gap between clinical and experimental data

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Scientific literature on the subject of probiotics has become particularly abundant in recent years. However, it appears that, for the digestive tube at least, there are two types of literature that seem to ignore each other completely. The first concerns the effects of probiotics on humans. In these works, the reasons for testing one microorganism rather than another are rarely explained, or the choice of the physiological impacts tested is not accounted for by preliminary tests on animals. The second type of literature concerns data resulting from basic experimental studies, performed *in vitro* or on animals, and that have never then been "put to the test" on humans.

A marked example of how the works run in parallel is provided by the anti-diarrhoeal effects of probiotics. Numerous studies show that treatment with some microorganisms is effective against antibiotic-associated diarrhoea, diarrhoea linked with acute gastro-enteritis and traveller's diarrhoea (Marteau *et al* 2001). A study of the literature on experimental data on the theme of probiotics and diarrhoea indicates that very few studies show that probiotics can influence water and electrolyte transport through the intestine. The study by Madsen *et al* (2001) shows that *in vitro* in an Ussing chamber, the intestines of interleukin-10 deficient mice have a short-circuit current value that is abnormally low, corresponding to damage to ion transport through the mucosa. These changes no longer appear when the mice are treated for one month with a probiotics mixture. Although studies on humans should necessarily be based on experimental work, it is clear that nobody would have dared start a study based merely on this one piece of data.

Another example is the effect of probiotics on the treatment of digestive functional disorders. There are very few studies in this field and some are very inconclusive, but it does appear that treatment with probiotics can improve the symptoms of such digestive disorders. The problem is that the main symptom is abdominal pain and it is very unclear how a microorganism can have antalgic properties. What is sure is that no experimental data indicates this. A closer look at the studies on digestive functional disorders indicates that the symptom the most often improved by probiotics is the feeling of bloatedness. It is easy to imagine that probiotics can reduce colic fermentation, but this still needs to be shown experimentally.

Other examples can be found of studies on human subjects that have not resulted from preliminary experimental data. We can indeed also ask ourselves what has been done, or could be done, in human studies as a result of some experimental data.

One reason for this parallel literature is simple and even encouraging - a probiotic is not a drug and "pre-clinical" studies must be different. Reducing diarrhoea induced in rats by an agent as drastic as ricin oil was at the start of the world's most popular anti-diarrhoea treatment and it is unlikely that a probiotic could be active in this type of diarrhoea. And yet, the anti-diarrhoeal properties of some probiotics have been clearly shown in humans.

If the effect of a probiotic has been established in humans, then knowledge of the action mechanisms from experimental studies done *in vitro* or in animals may appear superfluous - the essential thing being to improve human health. A comparison here with a medicine would be that, fortunately, we did not wait to understand the action mechanism of aspirin before using it.

Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. (2001). Protection from gastrointestinal diseases with the use of probiotics. *Am J Clin Nutr* 73 Suppl, 430S-436S.

Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. (2001). Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 121, 580-591.

## Efficacy of probiotics on persistent diarrhoea in young children

The effectiveness of probiotics in treating infant diarrhoea has been studied for some strains of probiotics including lactobacillus. A team from the CERELA, in Argentina, has specifically assessed the effect of consuming *Lactobacillus* sp. and *Saccharomyces boulardii* on persistent diarrhoea in children, something that had not been done before. The clinical trial was conducted randomised, double-blind and placebo-controlled on a population of hospitalised children (1).

A total of 89 children aged between 6-24 months took part in the study - 29 received a placebo, 30 milk supplemented with *S. boulardii* and 30 others milk supplemented with *L. acidophilus* and *L. casei*. The lyophilised micro-organisms were suspended in the milk and administered at doses of about  $1 \times 10^{11}$  cfu per day over 5 days.

The children receiving the lactobacilli or yeast gradually had less frequent stools than the control group. On the fifth day of treatment, the number of stools daily was  $2.0 \pm 2.0$  and  $1.5 \pm 0.9$  respectively versus  $5.2 \pm 3.0$  ( $p < 0.001$ ) for the placebo. In the same way, the time the diarrhoea lasted (in days) was shorter for the children given the probiotics  $3.7 \pm 1.3$  and  $3.8 \pm 1.5$  days respectively against  $8.5 \pm 4.2$  days ( $p < 0.005$ ) for the placebo. The length of time the symptoms (vomiting, abdominal pain, etc.) lasted was also significantly reduced in the children receiving the probiotics; the results obtained were similar with lactobacilli and yeast.

An infectious agent was isolated in 40 % of the patients, of whom 24 % were infected with rotavirus and the others with *E. coli*, *Salmonella* or *Shigella*. Whether the patients were infected with rotavirus or other bacterial species, the

lactobacillus and yeast were similarly effective ( $P < 0.1$ ).

At the end of treatment, the presence of *L. casei* was sought in the stools of the patients given the lactobacilli mixture. The strain was detected in 28 patients in quantities of  $1 \times 10^7$  cfu/g of stools.

The results of this study highlight the efficacy of *L. casei*, *L. acidophilus* and *S. boulardii* in treating persistent diarrhoea in children. The two strains of lactobacilli and yeast were equally effective, even in cases where infection was caused by rotavirus.

1• Gaon D, Garcia H, Winter L, Rodriguez N, Quintas R, Gonzalez SN, Oliver G (2003). Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhoea in children. Medicina (B Aires). 63(4):293-8.

## Probiotics fighting colon cancer - a preventive role ?

It is now accepted that the accumulation of heterocyclic amines (HCAs), that is formed from amino acids when food is cooked may be involved in the aetiology of human forms of cancer. Dietetic elements, such as fibres (2) and lactic bacteria (3), are now being sought whose intake could be inversely correlated to the incidence of colon cancer.

The goals of Knasmuller's team (4) were to assess a possible protective role of four lactic bacteria currently used in the production of fermented milks against the effects of HCAs. More precisely, the effect of *Streptococcus thermophilus* F4, *Streptococcus thermophilus* V3, *Lactobacillus bulgaricus* 291 and *Bifidobacterium longum* BB536 was assessed *in vivo* in rats by studying the prevention of DNA damage cause by HCAs (measured by the comet test).

The rats were force-fed a mixture of HCAs representative of those contained in grilled beef or chicken. Suspensions of each of the lactic bacteria were given

before (4-12 hours) or during HCA intake. The rats were sacrificed 4 hours after the carcinogen was administered and DNA damage was assessed in the colon and the liver.

Each of the four lactic bacteria, in quantities of  $1 \times 10^{10}$  bacteria, totally inhibited the DNA damage normally caused by beef HCA (to the hepatic or colic cells). The effect depends on the dose and remains measurable when  $1 \times 10^7$  lactic bacteria are administered or when the lactic bacteria were administered 12 hours before the HCAs. However, the same experiment performed on chicken HCAs had only a marginal, insignificant effect.

These results show that lactic bacteria play a real protective role against the genotoxic effects of some heterocyclic amines in the liver and the colon by preventing DNA damage. This gives grounds to suppose that consuming lactic bacteria could play a role in preventing cancer of the colon in humans. However, the

differences observed between the effects caused by beef HCAs (rich in quinoline and quinoxaline) and chicken HCAs (rich in Phip) show that the protective effect of lactobacillus depends on the composition of the HCA.

2• Jacobs DR Jr, Slavin J, Marquart L (1995). Whole grain intake and cancer: a review of the literature. Nutr Cancer. 24(3):221-9.

3• Wollowski I, Rechkemmer G, Pool-Zobel BL (2001). Protective role of probiotics and prebiotics in colon cancer. Am J Clin Nutr. 73(2 Suppl):451S-455S.

4• Zsivkovits M, Fekadu K, Sontag G, Nabinger U, Huber WW, Kundi M, Chakraborty A, Foissy H, Knasmuller S (2003). Prevention of heterocyclic amine induced DNA-damage in colon and liver of rats by different lactobacilli strains. Carcinogenesis. 24(12):1913-1918.

## Confirmation of the bifidogenous effect of *Bifidobacterium lactis* HN019

Among the dominant species in the intestinal microflora, lactobacilli and bifidobacteria are well known for their positive effects on humans (5). Consequently, an increase in the number of bifidobacteria and/or lactobacilli in the colon is a sought-after effect. Currently, two approaches are used to modulate the numbers of these bacteria. The probiotic approach consists in increasing the number of these microorganisms through food intake thanks to intake of fermented milks. The prebiotic approach lies in the consumption of foodstuffs that encourage the multiplication of such bacteria in the intestine ; these are generally oligosaccharides.

In a double-blind study performed with a placebo, Gopal et al (6) compared the impact on the intestinal flora of the consumption of *Bifidobacterium lactis* HN019 and galacto-oligosaccharide, a compound known to stimulate the growth of bifidobacteria.

Thirty volunteers were split into three groups that consumed 250 ml of milk supplemented either with *B. lactis* ( $3 \times 10^{10}$  cfu/day), or galacto-oligosaccha-

ride (2.4 g/day), daily for 4 weeks. Bacteriological analyses (search for anaerobic bacteria, bacteroids, enterobacteria, streptococci, bifidobacteria, lactobacilli and *Clostridia*) were performed on the faeces once a week for 8 weeks, commencing two weeks before the start of the test.

No changes in the composition of the intestinal flora were observed in the control group consuming just milk. Identical modifications were however observed in all the patients receiving supplemented milk - an increase in the number of bifidobacteria ( $p < 0.0002$ ) and lactobacilli ( $p < 0.004$ ) in the faeces. When intake was stopped, the number of bifidobacteria and lactobacilli returned to the level observed at the beginning of the test.

The authors also sought to determine the proportion of *B. lactis* HN019 in the population of faecal bifidobacteria. The proportion of this probiotic in the faeces was seen to differ greatly from one patient to another. The extreme values are 68.8 % and 0.1 % with a mean value of 28 %.

The results show that *B. lactis* HN019 is a strain capable of transiting live through the gastro-intestinal tract of humans and proliferating. This proliferation is transitory and ceases when intake stops. According to the authors, multiplication of the lactobacilli consumed could be due to the acidification of the pH in the colon caused by the increase in the population of bifidobacteria.

5• Mitsuoka T (1990). Bifidobacteria and their role in human health. *Indust Microbiol* 6:263-268.

6• Gopal PK, Prasad J, Gill HS (2003). Effects of the consumption of *Bifidobacterium lactis* HN019 (DR10TM) and galacto-oligosaccharides on the microflora of the gastrointestinal tract in human subjects. *Nutr Res* 23(10): 1313-28.

## Preventing abdominal infection with probiotics -

### an alternative to antibiotics

Some patients with serious illnesses can only be fed parenterally. This may cause atrophy of the intestinal mucosa, that itself will lead to a weakening of its barrier function and expose it to the risk of bacterial translocation and septicaemia. To avoid this risk, clinical protocols generally recommend treatment with antibiotics. An alternative approach would be to increase the population of bacteria unable to cross the mucosa and that remain to "upset" the pathogenic flora. This second strategy has been tested on rats by a Swedish research team (7).

Intra-abdominal infection was provoked in 36 rats by cecal ligation. Some of them had already been fed *Lactobacillus reuteri* R2LC enterally. The animals were sacrificed 12 or 24 hours after the surgery.

Bacteraemia was observed in 90 % of the control rats as opposed to 50 % of the treated rats ( $p < 0.05$ ). The increase of endotoxins circulating systemically in the control rats post-ligation was absent after 12 hours in the treated rats and reduced by 47 % after 24 hours. However, the administration of *L. reuteri* R2LC had no effect on the clearance of *E. coli* from the blood. This result suggests that the beneficial effects of the probiotic are not due to a stimulation of the phagocytosis function. Finally, the probiotic significantly stimulated intestinal motility ( $p < 0.05$ ).

This study shows that the enteral administration of *Lactobacillus reuteri* R2LC can protect against bacteraemia and endotoxemia in severe abdominal infection provoked in experimental conditions. Stimulation of intestinal motility

may explain in part the beneficial effect of the probiotic. These results suggest that the enteric administration of probiotics may constitute an alternative to treatment with antibiotics in the prevention of infections.

7• Thorlacius H, Nobaek S, Wang XD, Andersson R, Molin G, Bengmark S, Jeppsson B (2003). Lactobacilli attenuate bacteremia and endotoxemia associated with severe intra-abdominal infection. *Surgery* 134(3):467-73.

## The benefits of *Lactobacillus johnsonii* on *Helicobacter pylori* infections - a double-blind study

Research on the clinical effects of probiotics on humans has resulted in a wealth of literature. A particularly interesting field concerns the study of their efficacy in managing *Helicobacter pylori* infections. This bacterium is a pathogenic agent responsible for gastritis and peptic ulcers that may degenerate into gastric cancer. The results are encouraging but more clinical trials are needed. The use of fermented milk, as a supplementary treatment or as prevention, is one of the most promising lines of research (8).

Working with an industrial partner, researchers from the University Hospital of Lausanne carried out a double-blind, placebo-controlled, randomised clinical study on 50 adults infected by *H. pylori* but asymptomatic, to assess the efficacy of consuming fermented milk (9).

The volunteers were observed over 16 weeks, they were asked to consume 250 g of fermented milk (or a placebo) during the first 3 weeks and then 125 g for the next 13 weeks. The fermented milk (LC1) contained *Streptococcus thermophilus* and *Lactobacillus johnsonii* ( $1 \times 10^6$ - $1 \times 10^7$  cfu/g); the placebo contained only *S. thermophilus*.

*H. pylori* was not eradicated in either group, as is shown by the microbiological culture, histological examination and the breath urea test. The severity of the gastritis, appraised by comparing the cellular inflammation scores at the end of the study to those obtained beforehand ( $p=0.002$ ), was reduced in the antrum but not in the corpus. No changes to the inflammation score were observed in the control group in these two periods.

Treatment with LC1 caused an increase in the thickness of the mucous layer in both the antrum and the corpus, whereas it became thinner during the same period in the control patients. Intake of LC1 during 3 weeks reduced the amount of *H. pylori* in the antrum ( $p=0.04$ ), in comparison to the pre-treatment period, but not the amount in the corpus. This effect was maintained for the 16 weeks of the study. The density of *H. pylori* did not change in the control group receiving the milk containing *S. thermophilus* alone.

This study shows that a 4-month treatment with *S. thermophilus* and *L. johnsonii* in fermented milk causes a reduction in the amount of *H. pylori* and a

reduction in the severity of gastric inflammation caused by *H. pylori*, in particular in the antrum. This effect is maintained during the entire period of consumption. This effect appears to be due to the presence of *L. johnsonii* in fermented milk since it was not observed in those patients given fermented milk containing only *S. thermophilus*. Significantly, the fermented milk was not able to eradicate the pathogenic agent. However, regular intake of this fermented milk may be able to reduce the risk of the secondary complications of gastric inflammation.

8• Pantoflickova D, Corthesy-Theulaz I, Dorta G, Stolte M, Isler P, Rochat F, Enslen M, Blum AL (2003). Favourable effect of regular intake of fermented milk containing *Lactobacillus johnsonii* on *Helicobacter pylori* associated gastritis. *Aliment Pharmacol Ther.* 18(8):805-13.

9• Hamilton-Miller JM (2003). The role of probiotics in the treatment and prevention of *Helicobacter pylori* infection. *Int J Antimicrob Agents.* 22(4):360-6.

## *Lactobacillus gasseri* OLL2716 also effective in fighting *Helicobacter pylori* infections

Ushiyama et al (10) have assessed the efficacy of *L. gasseri* to fight a strain of *H. pylori* that is resistant to clarithromycin (CAM), an antibiotic often administered to treat this pathogen.

*In vitro* studies show that *L. gasseri* inhibits the growth of *H. pylori* whether it is susceptible or resistant to CAM. *In vivo* studies on axenic mice infected with CAM-resistant *H. pylori* confirm that CAM reduces the number of CAM-sensitive

*H. pylori* 8 weeks after infection ( $p<0.05$ ) but not the number of CAM-resistant *H. pylori*. The studies show that treatment with *L. gasseri* significantly reduces the number of pathogens whether or not they are CAM-susceptible or resistant.

The probiotic *L. gasseri* could be an attractive alternative for countering *H. pylori* infections, in particular when this pathogen resists to antibiotics treat-

ment. Clinical trials, properly performed on human subjects, are now required to confirm these hopes.

10• Ushiyama A, Tanaka K, Aiba Y, Shiba T, Takagi A, Mine T, Koga Y (2003). *Lactobacillus gasseri* OLL2716 as a probiotic in clarithromycin-resistant *Helicobacter pylori* infection. *J Gastroenterol Hepatol.* 18(8):986-991.

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

## Lactic acid bacteria capable of vaccinating mice against allergies

Recent clinical results highlight the potential role of lactic bacteria in allergy prevention (11). As part of the search for methods to prevent and treat allergic manifestations, a team has assessed the immuno-modulating ability of *Lactococcus lactis* and *Lactobacillus plantarum* to fight birch pollen allergies in mice (12).

*In vitro*, each of the lactic bacteria was able to stimulate the production of IL-12 and IFN $\gamma$  (Th1 type response) cytokines in the naive spleen cells of mice. Cytokine production is dependent on the quantity of lactic bacteria. However, unlike *E. coli* stimulation, the production of Th2\* type cytokines is little or not at all stimulated by the lactic bacteria.

The intra-nasal injection of the birch pollen allergen together with one or

other of the lactic bacteria before allergic sensitisation (provoked by intra-peritoneal injection of the allergen) caused an increase in specific IgG2a antibodies in the serum and an increase in the production of IFN $\gamma$  by the spleen T cells isolated *in vitro* and stimulated by the allergen. These results indicate a shift towards a Th1 type immune response. The results are comparable if the allergen and the lactic bacteria are injected after sensitisation.

This study shows that joint nasal administration of a birch pollen allergen and *L. lactis* or *L. plantarum*, before or after allergic sensitisation, causes an immune response capable of blocking allergic type responses. This immuno-modulatory effect of the lactic bacteria appears to be due to their intrinsic ability to promote a Th1 type response via the production of IL-12 and IFN $\gamma$ . These

results show that a protocol to use lactic bacteria to prevent and treat allergies can be envisaged.

\* The allergic reaction is regularised by T-helper (Th) lymphocytes. The Th2 subclass appears to be responsible for the allergic response via the production of cytokines, in particular IL-4 and IL-5. Th1 lymphocytes, on the other hand, would appear to counter Th2 type responses via the production, among others, of IL-12 and IFN $\gamma$ . The appearance of the allergy will therefore depend on the balance between the Th1 and Th2 type responses.

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## Lactic bacteria able to modulate the inflammatory response

### via their DNA

Recent works have shown that DNA sequences rich in unmethylated CpG motives and enriched in bacterial DNA, have an immuno-modulating ability (13). Given that *Bifidobacterium* and, to a lesser extent, *Lactobacillus* have a high content of CpG motives, Campieri et al (14) have examined the effect of the DNA of these bacteria on the immune response. The VSL#3\* preparation was chosen since this mixture of probiotics appears to have a salutary effect on intestinal inflammation (15, 16).

Two types of experiment were conducted. One consisted in using purified DNA from each of the bacteria in VSL#3 to induce the production of cytokines by peripheral blood mononuclear cells. The second consisted in performing this stimulation with bacterial DNA isolated from the faeces of healthy human subjects both before and after they had consumed VSL#3 for 2 weeks.

The DNA of each of the VSL#3 bacteria induced *in vitro* the production of IL-1, IL-6 and IL-10 interleukins. Production depended directly on the quantity of DNA placed in contact with the lymphocytes and varied according to the strain. *Bifidobacterium* and *S. thermophilus* are IL-10 inducers at levels about three times greater than the liposaccharide used as a positive control. The DNA isolated in the faeces before consumption of VSL#3 induced greater concentrations of IL-1 $\beta$  than IL-10; however, the DNA isolated after intake of VSL#3 induced greater secretion of IL-10 than IL-1 $\beta$ .

These results show that the DNA of the probiotics tested can stimulate the production of the anti-inflammatory cytokine IL-10 and that this property is retained during human consumption of these probiotics. This data suggests that the DNA of some lactic bacteria could stimulate the production of IL-10 in the intestine and consequently modulate the

inflammatory response. This property, whose mechanism is not yet understood, may be an argument for using probiotics to treat inflammatory intestinal disease.

\* VSL#3 is a probiotic mix containing *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus bulgaricus*.

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