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SCIENTIFIC SURVEY . LACTIC ACID BACTERIA . PROBIOTICS

IN THIS ISSUE

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Probiotics - more specific and effective ways to combat abnormalities in the intestinal microbiota?

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Probiotic research has been successful both in intervention studies and in food product development. Today, probiotics provide the majority of functional foods. They have been defined by the ILSI Europe (International Life Sciences Institute Europe) working group as "a viable microbial food supplement which beneficially influences the health of the host". In 2002, this definition was further refined by the FAO/WHO expert group to include the required dose. Both definitions require the efficacy and safety of the micro-organism to be scientifically demonstrated. Selection criteria for future probiotics will focus on specific criteria, but will still include traditional adhesion studies and acid and bile tolerance. Demonstration of health effects and benefits requires research on action mechanisms and clinical intervention studies with human subjects belonging to the target groups.

Today, a wealth of scientific studies on probiotics, including both intervention and other studies, is available in the literature. Data on potential health effects has increased and facilitates our understanding of the interactions between specific probiotics and the intestinal microbiota. Several questions remain to challenge the researchers. These include the following:
- Can smaller probiotic doses be administered to infants and children, than to adults whose microbiota is stable, diverse and more complex?
- Are larger doses and longer treatment times required for elderly subjects with a stable microbiota?
- How important is viability for health effects? How is viability determined (using plate counts or more sensitive methodologies) and can viable but not culturable (dormant) probiotics bring health benefits?
- Can the safety of the new effective probiotics be guaranteed during development?

All these questions require definite answers that will be provided by the development of new methods, but they will also have a significant regulatory impact.

What is new and significant in the area of probiotics today? In my opinion, verifying clinical effects through intervention studies needs to be continued and coupled with novel molecular methodologies to evaluate health effects. More studies of probiotic and prebiotic action mechanisms are required. Clinical research can play an important role, first by characterising abnormal microbiota in human subjects with health problems and then by defining probiotics to counteract the abnormalities or to prevent the establishment of deviations in the microbiota. Such an approach is urgently needed.

Maintaining good health through probiotics is a new target that could lead to selecting specific strains to reduce the risk of particular diseases in specific populations. Such information will redefine the way we select, characterize and assess future probiotics and prebiotics. Such data will also enable us to take into account the genetic factors that influence the performance of probiotics in industrial processes and products. The comparative genomics of probiotics and other members of the intestinal microbiota will provide valuable information, shedding light on the functional properties of probiotics and their safety. This will increase our understanding of their biological mechanisms and will be an important step in our understanding of human biology.

The most important issue is that we must still provide food products that are "tasty" for the consumer. If this can also be achieved, science, the food industry and the consumer will all be satisfied - to the mutual benefit of all.

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A lactobacillus against colorectal tumours

a clinical trial requiring further confirmation

The existence of a correlation between diet and the risk of intestinal cancer is well-established today. Those who eat only small quantities of fruit and vegetables, for example, are defined as being at high risk. The consumption of probiotic bacteria would appear to avert the risk of tumours in the digestive tract – an effect that has already been seen in animals (1). A study focusing on *Lactobacillus casei Shirota* assessed the effect of consuming probiotics in a human population considered high risk for the development of colorectal tumours (2).

380 patients who had had at least 2 colorectal tumours removed were divided into four groups, and given a diet consisting of one of the following products: biscuits containing bran (7.5 g per day), the bacterium *L. casei Shirota* (taken in capsules, $1x10^{10}$ cfu after each meal), both products simultaneously or neither of the two products (control group). The treatment was followed over 4 years and was known both by the patients and the medical staff. The appearance of new tumours after 2 and then 4 years was diagnosed by colonoscopy.

In comparison with the control group, intake of the tested products did not result in any significant differences as regards the appearance of new tumours. However, differences were seen in the type of tumours observed. In the patients who were given the bran (2 groups out of 4) the number of patients with large tumours after 2 years was significantly higher than in the other groups. This result was not seen after 4 years. In the patients given the *L. casei* (2 groups out of 4), the occurrence of tumours with a grade of moderate atypia (i.e. suspect) was significantly lower than in the other patients.

Although the number of relapses was not affected, consumption of *L. casei Shirota* for 4 years after the resection of colorectal tumours reduced the proportion of suspect tumours. A surprising result was that the consumption of bran increased the size of the tumours; this effect was observed only after 2 years and not after 4 years. These results show the potential advantages of probiotics in managing the risk of colorectal cancer and appear to contradict the consensus that dietary fibres have a beneficial effect on cancer of the colon. Despite the large number of patients taking part in the study, the forcefulness of the results suffers from the fact the study was not placebo controlled or conducted double blind (1).

1• **Donaldson MS (2004).** Nutrition and cancer: a review of the evidence for an anti-cancer diet. Nutr J 20, 3-19.
2• **Ishikawa H, Akedo I, Otani T, Suzuki T, Nakamura T, Takeyama I, Ishiguro S, Miyaoka E, Sobue T, Kakizoe T (2005).** Randomized trial of dietary fiber and Lactobacillus casei administration for prevention of colorectal tumors. Int J Cancer 116, 762-767.

The bacteria found in yoghurt play a direct role

in modulating the risk markers of colon cancer

In mice, Gabriela Perdigon's team has highlighted a possible method of interaction between yoghourt bacteria and colic tumours (3).

In a 6 month experimental protocol, 6 groups of 45 to 50 mice were subjected to different treatments. Tumours were induced in two groups. In one group a colic tumour was induced chemically and no yoghurt was given, in the other group, 10 days of yoghourt administration were followed by the induction of a tumour and a cyclic consumption* of yoghourt from week 8. Four other groups did not have tumours induced: one group was given yoghurt in cycles from week 8, in another, yoghourt supernatant fluid was given in cycles from week 8, in a third, skimmed milk was given in cycles from week 8 and finally a last group was given no products and acted as the control group.

The yoghurt was a milk fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The yoghurt supernatant fluid was obtained by centrifuge, it did not contain bacteria. The effect of each treatment on the activity of two procarcinogenous enzymes, β- glucuronidase and nitroreductase, was measured in the colon on a monthly basis.

In the healthy mice, the activity of the two enzymes increased with age. In these mice, consuming the yoghurt caused enzymatic activity that was similar or less than was observed in the untreated control group and in the groups receiving the yoghurt supernatant fluids or the milk.

Administering the carcinogen led to an increase in the activity of the two procarcinogenous enzymes. The consumption of yoghurt resulted in a reduction of this enzymatic activity compared to the group with tumours that did not consume yoghurt.

These results show the action mechanism of yoghurt consumption on colic tumours. Yoghurt reduces the activity of procarcinogenous enzymes in healthy mice or mice carrying tumours. As this effect is only seen when the entire yoghurt – and not the yoghurt supernatant fluid obtained by centrifuge – was administered, the yoghurt bacteria is probably directly involved in this mechanism.

* The oral administration of the different products consisted in alternating 10 days with the product and 7 days without.

3• **de Moreno de LeBlanc A, Perdigon G (2005).** Reduction of beta-glucuronidase and nitroreductase activity by yoghurt in a murine colon cancer model. Biocell 29, 15-24.

Probiotics protect against necrotizing enterocolitis in neonates

Necrotizing enterocolitis (NEC) is an infection seen in newborn babies that takes the form of an intestinal attack that may lead, in certain cases, to intestinal perforation and even death. This disorder particularly affects premature babies of very low birth weight. It may be linked to early colonization of the intestines by different germs (viruses, fungus, bacteria) (4). The use of probiotics to guard against the risk of infection has been envisaged. A study of a probiotic mix has shown the existence of such a preventive effect (5).

145 healthy premature neonates with a birth weight of less than 1 500 g were enrolled in the study as soon as oral feeding was started. They were distributed randomly and blind between a group receiving – during mealtimes and for 36 weeks – a probiotic mix (total of $1.05x10^9$ cfu/day diluted in the food, containing $0.35x10^9$ cfu of each of the following strains – *Bifidobacterium infantis*, *Streptococcus thermophilus* et *Bifidobacterium bifidus*) and a group that received no probiotics. The severity of the NEC was noted according to Bell's criteria – a three-stage clinical scale from the least to the most severe (6).

Consumption of the probiotic mix caused no side effects; all the infants developed in similar fashion. In comparison with the control group, intake of the probiotic mixture was accompanied by a significant reduction in the occurrence and severity of NEC (occurrence 4% vs. 16.4%, p=0.03; severity according to Bell's clinical staging 1.3 ± 0.5 vs. 2.3 ± 0.5 p=0.005). Furthermore, the three deaths reported during the study occurred in the control group.

Administering a probiotic mix to premature infants of very low birth weight led to a reduction in both the occurrence and severity of cases of NEC. This thorough study, conducted randomly and blind on a significant number of patients, is an argument for the preventive use of probiotics in premature infants.

4• **Claud EC, Walker WA (2001).** Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J 15, 1398-1403.
5• **Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, Hammerman C (2005).** Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 147, 192-196.
6• **Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, Brotherton T (1978).** Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg 187, 1-7.

A lactobacillus able to cure diarrhoea in neonates

Some probiotics have been seen to be effective in treating diarrhoea of viral origin in children. In a study by Szajewska et al. (a randomized, double-blind clinical study), of 81 children aged between 1 and 36 months, the administration of *Lactobacillus GG* during the entire time spent in hospital reduced the number of cases of rotavirus gastroenteritis (7).

A study carried out in Bangladesh has added new information concerning the scope of action of probiotics on infant diarrhoea (8). It was also a randomized, double-blind study. 230 infants aged from 4 to 24 months suffering from diarrhoea for less than two days, received daily and for 5 days, either a placebo, or $1x10^{10}$ cfu of *Lactobacillus paracasei ST11* diluted in an oral rehydration solution. Stool output and frequency, oral rehydration solution intake, and excretion of rotavirus were monitored daily.

The probiotic had no effect on patients suffering from severe rotavirus diarrhoea. However, compared to the patients receiving the placebo, *L. paracasei ST11* significantly reduced ($p<0.05$ in each case) the quantity and frequency of the stools and the intake of the oral rehydration solution in children suffering from non-severe non-rotavirus diarrhoea. In these children, the diarrhoea was completely cured by the end of the treatment in 76% of the patients receiving the probiotic compared to 49% of the control group (statistically significant, p=0.03).

In conclusion, the probiotic had a beneficial effect on patients suffering from diarrhoea that was not severe and not caused by rotavirus, whereas no impact on severe rotavirus diarrhoea was observed. However, despite a well-constructed protocol, the available data does not make it possible to conclude whether the beneficial effect of the probiotic can be associated with the slight severity of the diarrhoea or the fact it was not caused by rotavirus. Additional studies will therefore be necessary to target precisely the types of diarrhoea likely to be improved by this probiotic.

7• **Szajewska H, Kotowska M, Mrukowicz JZ, Armanska M, Mikolajczyk W (2001).** Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. J Pediatr 138, 361-365.
8• **Sarker SA, Sultana S, Fuchs GJ, Alam NH, Azim T, Brussow H, Hammarstrom L (2005).** Lactobacillus paracasei strain ST11 has no effect on rotavirus but ameliorates the outcome of non rotavirus diarrhea in children from Bangladesh. Pediatrics 116, 221-228.

L.casei fermented milk plays a role in *H. pylori* eradication in children

The role played by *Helicobacter pylori* in the aetiology of dyspepsia is much debated, however the eradication of this pathogenic bacterium is recommended in infected children suffering from gastro-intestinal disorders (9). Certain probiotics are active *in vitro* against *H. pylori*, and it is planned to use them as an adjuvant to a conventional course of antibiotics. In asymptomatic adults, studies have shown that probiotics increase tolerance to antibiotic treatments but are ineffective in eradicating the pathogen (10). For the first time, a study of dyspeptic children has shown an increased efficacy of the medical treatment when it is supplemented by the consumption of fermented milk (11).

Eighty children of an average age of 12 years were recruited. They were all infected with *H. pylori* and were suffering from severe dyspepsia. In addition to the conventional eradication treatment (7-day tritherapy), the patients received, randomly and double blind over 14 days, either pasteurised milk or milk fermented with yoghurt bacteria and *Lactobacillus casei DN-114 001* ($1x10^{10}$ cfu *L. casei* / day). The efficacy of the treatment was assessed after 4 weeks by two non-invasive *H. pylori* detection tests.

The eradication rate in the control group was 61.3%; in the group that had been given the fermented milk, it reached 91.6%. The difference between the two groups was statistically significant (p=0.0019). The occurrence of side effects was slight and similar in both groups.

In symptomatic children, eradication of *H. pylori* by a conventional treatment was increased thanks to the simultaneous intake of *L.casei*. This clinical study shows that probiotics can help improve not simply the tolerance to but also the efficacy of an antibiotic treatment. This result raises new questions. Are the effects of fermented milk in eradicating *H. pylori* specific to children? Are they due to a single probiotic strain or a bacteria mixture? Other investigations would be required to answer these questions.

9• **Drumm B, Koletzko S, Oderda G, et al (2000).** Helicobacter pylori infection in children: report of a consensus conference held in Budapest, September 1998. J Pediatr Gastroenterol Nutr. 39, 246-252.
10• **Myllyluoma E, Veijola L, Ahlroos T, Tynkkynen S, Kankuri E, Vapaatalo H, Rautelin H, Korpela R (2005).** Probiotic supplementation improves tolerance to Helicobacter pylori eradication therapy - a placebo-controlled, double-blind randomized pilot study. Aliment Pharmacol Ther 15, 1263-1272.
11• **Sykora J, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, Varvarovska J, Stozicky F, Pazdiora P, Schwarz J. (2005).** Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. J Clin Gastroenterol 39, 692-698.

A probiotic can reduce high blood pressure in humans

One of the supposed action mechanisms of probiotics is the production of functional factors. In particular, production by certain bacteria of angiotensin I-converting enzyme inhibitors from milk has already been reported (12). In a recent study of human patients, consuming such factors was seen to moderate blood pressure (13).

The tested product was a milk fermented by *Lactobacillus* containing two angiotensin-I peptide inhibitors and administered as milk powder in tablet-form. Eighty patients, of an average age of 51, were recruited. Half of them had high-normal blood pressure and the others mild hypertension (grade 1). Within each group, the patients were divided randomly and double blind into sub-groups that, on a daily basis and for 4 weeks, were given either the tested product or a placebo. The authors analyzed changes in the systolic or diastolic blood pressure.

Once product administration had ceased, in the patients with high-normal blood pressure the diastolic blood pressure had reduced significantly (-5,0 mm Hg, p=0,04). In the group given the fermented milk as compared to the group given the placebo; the systolic blood pressure showed a non-significant reduction. In the patients with mild hypertension, the systolic blood pressure was significantly lower to that observed in the placebo group (-11.2 mm Hg, p=0.003) and the diastolic blood pressure showed a non-statistically significant reduction. No side effects were reported during the study.

Consumption of dehydrated fermented milk was effective in reducing blood pressure in slightly hypertensive patients. Such products could play a role in treating mild hypertension by providing a simple, non-medicinal strategy that requires no change in eating habits. Additional studies of the products are however necessary given the modest nature of the results (only one of the two blood pressure measurements changed statistically in each case). The efficacy of the products should also be studied over a longer period, since strategies for fighting high blood pressure are necessarily long-term.

12• **Nakamura Y, Yamamoto N, Sakai K, Okubo A, Yamazaki S, Takano T (1995).** Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. J Dairy Sci 78, 777-783.
13• **Aihara K, Kajimoto O, Hirata H, Takahashi R, Nakamura Y (2005).** Effect of powdered fermented milk with Lactobacillus helveticus on subjects with high-normal blood pressure or mild hypertension. J Am Coll Nutr 24, 257-65.

Testing a cocktail of probiotics, vitamins and minerals to fight winter infections in humans

Via their effect on immunity, it is expected that probiotics may help prevent certain infections. A study on adults has brought new elements to the fore (14).

477 adults in good health, of an average age of 36, were given randomly either a placebo or a dietary supplement containing vitamins, minerals and probiotics (total of $5x10^8$ cfu/day, strains of *Lactobacillus gasseri PA 16/8*, *Bifidobacterium longum SP 07/3* and *Bifidobacterium bifidum MF 20/5*). The administration period, either 3 or 5.5 months was situated between December and June. Occurrence of respiratory tract infections and their associated symptoms were estimated from the questionnaires completed by the patients. With an objective of only studying infections of viral origin, attacks leading to the prescription of antibiotics were excluded. The cellular immune parameters were studied before and after 14 days of consuming the tested product in 60 patients chosen randomly from each group.

The incidence of respiratory infections of viral origin in the patients given the probiotics was reduced by 13.6% which was statistically insignificant (p=0.07) compared to the control group. The observed symptoms were reduced by taking probiotics, with, in particular, a reduction relative to the control group, of 19% for the total symptoms score p=0.12), of 25% for flu symptoms (p=0.09), and 54% for the number of days with fever (p=0.03), and 30% for headaches (p=0.04). The length of the infection did not alter. The total quality of leucocytes, lymphocytes and monocytes between the start of the study and the 14th day of product consumption was significantly higher in the group given probiotics than in the control group.

It would seem that, in relation to a placebo, when adult humans consumed a dietary supplement containing probiotics, this led to a reduction in the severity of certain symptoms of viral infections of the respiratory tract. This effect could be linked to a reinforcement of cellular immunity. The study certainly included a large number of patients (477), a key criterion for judging the infallibility of the results, but it should be remembered that it was the patients themselves that graded their symptoms and that the study gave no indication of the relative roles of the various components making up the supplement: probiotics, vitamins and minerals. In other words, the question is to know whether the probiotics played a significant role compared to the vitamin and mineral supplements.

14• **Winkler P, de Vrese M, Laue Ch, Schrezenmeir J (2005).** Effect of a dietary supplement containing probiotic bacteria plus vitamins and minerals on common cold infections and cellular immune parameters. Int J Clin Pharmacol Ther 43, 318-326.

This scientific letter **"Yoghurts & fermented milks"** is also available on the following website:
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Confirmation that yoghurt cultures survive during intestinal transit in humans

Since the beneficial effect for the host is linked to the metabolic activity of the probiotic, its survival in the digestive tract is a prerequisite to its efficacy. Therefore, the bacteria found in yoghurt (*Lactobacillus bulgaricus* et *Streptococcus thermophilus*) must remain alive to have a beneficial effect on lactose digestion. A study of 13 healthy volunteers has shown that yoghurt consumption in quantities of 375 ml per day results in viable bacteria in the faeces in 82% of cases for *S. thermophilus* and 95% of cases for *L. bulgaricus*.

15• **Mater DD, Bretigny L, Firmesse O, Flores MJ, Mogenet A, Bresson JL, Corthier G (2005).** Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus survive gastrointestinal transit of healthy volunteers consuming yogurt. FEMS Microbiol Lett 250, 185-187.

Action mechanism of *Lactobacillus casei DN-114 001* against intestinal inflammation

In patients suffering from Crohn's disease, enteroinvasive bacteria colonize intestinal lesions caused by the autoimmune reactions behind secondary inflammation. Neutralizing these pathogens by blocking their access to the intestinal cells could avoid or diminish inflammatory attacks. *In vitro*, the strain *Lactobacillus casei DN-114 001* significantly inhibited the adhesion and invasion of human intestinal cells by different strains of enteroinvasive *Escherichia coli* taken from patients suffering from Crohn's disease. This effect is strengthened by the presence of *L. casei* supernatant fluids. It is therefore reasonable to believe that lactobacillus is responsible for the production of a factor active against adhesion.

16• **Ingrassia I, Leplingard A, Darfeuille-Michaud A (2005).** Lactobacillus casei DN-114 001 inhibits the ability of adherent-invasive Escherichia coli isolated from Crohn's disease patients to adhere to and to invade intestinal epithelial cells. Appl Environ Microbiol 71, 2880-2887.

Failure of *Lactobacillus GG* used as an adjuvant in treating Crohn's disease in children

Probiotics are seen as potential supplements to medicinal treatments for intestinal inflammation. However, a study in children ended in failure. In clinical trial carried out randomly and double-blind, on 75 children suffering from Crohn's disease and in remission, consumption of *Lactobacillus GG* in addition to their standard treatment over two years did not significantly change either the occurrence of relapses or the length of remission periods compared to a placebo.

17• **Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL (2005).** A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm Bowel Dis 11, 833-839.

Yoghurt bacteria are probiotics

In a summary article, international experts have analyzed the background to the probiotic concept and presented the current official European Union definition. They remind us that beneficial effects on humans are pre-eminent above all other criteria in qualifying a product as a probiotic. Basing their arguments on a review of the major articles published on the subject, the authors reaffirm that yoghurt cultures deserve to be considered as probiotics thanks to their beneficial effect on digestion and the assimilation of lactose.

18• **Guarner F, Perdigon G, Corthier G, Salminen S, Koletzko B, Morelli L (2005).** Should yoghurt cultures be considered probiotic? Br J Nutr 93, 783-786.

What is revealed by the *Streptococcus thermophilus* genome

The comparison of the genome of three strains of *Streptococcus thermophilus* with the genomes of pathogenic streptococcus have given new insights into this bacterium that is widely used in the dairy industry. In comparison with pathogenic streptococcus, *S. thermophilus* has lost several virulence-related functions, including the ability to adhere to the cell wall. The species *S. thermophilus* is undergoing a process of regressive evolution towards a specialised bacterium for growth in milk. The nitrogen metabolism is well developed whereas the sugar metabolism has regressed, except for its adaptation to lactose as its main source of carbon.

19• **Hols P, Hancy F, Fontaine L, Grossiord B, Prozzi D, Leblond-Bourget N, Decaris B, Bolotin A, Delorme C, Dusko Ehrlich S, Guedon E, Monnet V, Renault P, Kleerebezem M (2005).** New insights in the molecular biology and physiology of Streptococcus thermophilus revealed by comparative genomics. FEMS Microbiol Rev 29, 435-463.

20• **de Moreno de LeBlanc A, Matar C, LeBlanc N, Perdigon G (2005).**
Effects of milk fermented by Lactobacillus helveticus R389 on a murine breast cancer model. Breast Cancer Res 7, R477-486.

21• **Dotan I, Rachmilewitz D (2005).**
Probiotics in inflammatory bowel disease: possible mechanisms of action. Curr Opin Gastroenterol 21, 426-30.

22• **Garrido D, Suau A, Pochart P, Cruchet S, Gotteland M (2005).**
Modulation of the fecal microbiota by the intake of a Lactobacillus johnsonii La1-containing product in human volunteers. FEMS Microbiol Lett 15, 249-56.

23• **Gueimonde M, Noriega L, Margolles A, de los Reyes-Gavilan CG, Salminen S (2005).**
Ability of Bifidobacterium strains with acquired resistance to bile to adhere to human intestinal mucus. Int J Food Microbiol 15, 341-6.

24• **Humblot C, Bruneau A, Sutren M, Lhoste EF, Dore J, Andrieux C, Rabot S (2005).**
Brussels sprouts, inulin and fermented milk alter the faecal microbiota of human microbiota-associated rats as shown by PCR-temporal temperature gradient gel electrophoresis using universal, Lactobacillus and Bifidobacterium 16S rRNA gene primers. Br J Nutr 93, 677-84.

25• **LeBlanc JG, Burgess C, Sesma F, de Giori GS, van Sinderen D (2005).**
Lactococcus lactis is capable of improving the riboflavin status in deficient rats. Br J Nutr 94, 262-7.

26• **Lewis SJ, Burmeister S (2005).**
A double-blind placebo-controlled study of the effects of Lactobacillus acidophilus on plasma lipids. Eur J Clin Nutr 59, 776-780.

27• **Matsumoto S, Hara T, Hori T, Mitsuyama K, Nagaoka M, Tomiyasu N, Suzuki A, Sata M (2005).**
Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. Clin Exp Immunol 140, 417-426.

28• **Ohno H, Tsunemine S, Isa Y, Shimakawa M, Yamamura H (2005).**
Oral administration of Bifidobacterium bifidum G9-1 suppresses total and antigen specific immunoglobulin E production in mice. Biol Pharm Bull 28, 1462-6.

29• **Park JH, Seok SH, Cho SA, Baek MW, Lee HY, Kim DJ, Chung MJ, Kim SD, Hong UP, Park JH (2005).**
Antimicrobial effect of lactic acid producing bacteria culture condensate mixture (LCCM) against Salmonella enteritidis. Int J Food Microbiol 101, 111-117.

30• **Plummer SF, Garaiova I, Sarvotham T, Cottrell SL, Le Scouiller S, Weaver MA, Tang J, Dee P, Hunter J (2005).**
Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. Int J Antimicrob Agents 26, 69-74.

31• **Pochard P, Hammad H, Ratajczak C, Charbonnier-Hatzfeld AS, Just N, Tonnel AB, Pestel J (2005).**
Direct regulatory immune activity of lactic acid bacteria on Der p 1-pulsed dendritic cells from allergic patients. J Allergy Clin Immunol 116, 198-204.

32• **Rinne M, Kalliomaki M, Arvilommi H, Salminen S, Isolauri E (2005).**
Effect of probiotics and breast-feeding on the bifidobacterium and lactobacillus/enterococcus microbiota and humoral immune responses. J Pediatr 147, 186-91.

33• **Sherman PM, Johnson-Henry KC, Yeung HP, Ngo PS, Goulet J, Tompkins TA (2005).**
Probiotics reduce enterohemorrhagic Escherichia coli O157:H7- and enteropathogenic E. coli O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. Infect Immun 73, 5183-5188.

34• **Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, van Capel TM, Zaat BA, Yazdanbakhsh M, Wierenga EA, van Kooyk Y, Kapsenberg ML (2005).**
Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J Allergy Clin Immunol 115, 1260-1267.

35• **Viljanen M, Pohjavuori E, Haahtela T, Korpela R, Kuitunen M, Sarnesto A, Vaarala O, Savilahti E (2005).**
Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. J Allergy Clin Immunol 115, 1254-9.

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