Yoghurts & fermented milks

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SCIENTIFIC SURVEY . LACTIC ACID BACTERIA . PROBIOTICS

IN THIS ISSUE

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Probiotics and the intestinal microbial ecosystem

Robert Ducluzeau

Is the ingestion of probiotics of fermented milk products containing live bacteria capable of modifying the microbial ecosystem of the human digestive tract? Studies confirm it and even add that this modification has a positive effect on the host. Their action on the flora's equilibrium would appear to be one part of the effects of the beneficial role of probiotics. However, the reality is certainly not quite so simple.

To highlight the action of probiotics on the flora, we need to have accurate knowledge of which bacterial groups are found there and in particular which ones make up the dominant flora of the digestive tract and which exert the main effects. The use now made of molecular biology techniques means knowledge of the flora of the lower human digestive tract has advanced considerably, but at the same time it pinpoints the huge gaps in knowledge that still need to be filled.

We can already affirm that 50 % of the dominant faecal microbial flora in humans cannot be cultivated in anaerobic conditions. We know, too, thanks to molecular biology methods, that 80 % of the species making up the dominant flora in healthy humans are not included in our current collections of strains. It is therefore clear how difficult it is to study the variations in a flora of which at least half is still unknown to researchers.

Paradoxically, it appears that the dominant faecal flora in humans is only composed of three phylogenetic groups, each of them however being represented by a large number of bacterial species. This diversity of species is specific to each individual and relatively stable over time. In short, we can say that each individual hosts a unique flora, determined more by the genetics of the host and by ecology than by variations in the environment and in particular food intake. This strong individual diversity in the composition in species of the faecal flora can be contrasted with a strong homogeneity in its global metabolic activity, so that, despite the differences in composition, the flora exerts the same positive effects on health in the majority of individuals. Under these conditions, it is difficult to imagine how the simple ingestion of a bacterial population, much fewer in number than that of the dominant populations of the digestive tract, can be sufficient to change the equilibrium of these in a way that is measurable by current techniques.

It can be seen that we are now far removed from the simplistic views that were at the origin of the development of the concept of probiotics. First and foremost, it should be noted that almost all scientists are in agreement that no strain of probiotic is capable of settling in the human digestive tract, i.e. of multiplying there and becoming a component of the ecosystem that is active on the host. Modifications to the faecal flora following the ingestion of probiotics have until now been observed using the classic techniques of anaerobic culture. Although modifications do occur and are reproducible, they still concern sub-dominant bacterial populations that are easy to cultivate but for which it is impossible to affirm that they have an impact on the health of the host. For the moment we are only able to confirm that live bacteria can be active during their transit through the small intestine where no real stable ecosystem exists. However, it is obvious that our knowledge is insufficient to affirm that the ingestion of live bacteria can modify the equilibrium of the dominant flora of the lower regions of the digestive tract. Bacterial ecologists still have a lot of work to do!

Brigidi P, Vitali B, Swennen E, Bazzochi G, Matteuzzi D (2001). Effect of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. Res Microbiol 152, 735-741.
Canzi E, Casiraghi M, Zanchi R, Gandolfi R, Ferrari A, Brighenti F, Bosia R, Crippa A, Maestri P, Vesely R, Bianchi Salvadori B (2002). Yoghurt in the diet of elderly: a preliminary investigation into the effect of the gut ecosystem and lipid metabolism. Lait 82: 713-723.
Doré J, Rigottier-Gois L (2004). Méthodes d'étude in "Flore Microbienne intestinale, Physiologie et pathologie digestive", Biocodex Ed., Paris.
Fujiwara S, Seto Y, Kimura A, Hashiba H (2001). Establishment of orally-administered Lactobacillus gasseri STB2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. J Appl Microbiol 90, 343-352.

Probiotics in infants' feeding

Today, the inclusion of probiotics – essentially bifidobacteria, streptococcus and lactobacillus – in the feeding of babies and infants is provoking great interest. Infant milk formula containing probiotics is now available on the market. The interest is motivated by the search for simple solutions to treat and/or prevent the gastro-intestinal infections affecting this group in particular.

This step is based on two observations. The type of feeding – mother's milk or formula – has an influence on the makeup of the faecal flora (1) – the predominance of bifidobacteria and lactobacillus in babies nourished on mother's milk; heterogeneous flora in those receiving formula (2). Breast fed babies are less sensitive to gastro-intestinal infections than children receiving formula (3). These results have led to the hypothesis that the massive presence of lactobacillus in the intestinal flora protects young children better against gastro-intestinal infections.

Several controlled clinical studies have shown that the administration of certain probiotics reduces the duration of infectious diarrhoea. Other studies have researched the existence of a preventive effect of probiotics against infectious diarrhoea and positive results have been obtained with *Lactobacillus* GG (4) and *Lactobacillus casei DN114001* (5). Other probiotics may also be effective. Feeding with a powdered milk preparation enriched with lyophilised *Bifidobacterium lactis* Bb12 has been shown to reduce the occurrence of diarrhoea and shedding of rotavirus in infants in residential care (6). These results have also recently been confirmed in France among healthy infants (7).

This French study, carried out double-blind and placebo-controlled, involved 90 healthy children (aged <8 months) across several different centres – child-care centres or nurseries. Two groups were formed. The control group received a standard formula product. The other group was given an acidified milk (fermentation with *S. thermophilus* and *L. helveticus* before lyophilisation) and addition of *Bifidobacterium lactis* Bb12 in quantities of $1x10^6$ cfu/g of powder (i.e. $1.5x10^5$ cfu/ml of reconstituted milk). The infants were given this diet for about 4 months.

The clinical study showed that fewer infants given the probiotic in their milk suffered from diarrhoea that the control infants (28.3 % of infants vs. 38.7 %) and that the diarrhoea lasted a shorter period of time in these children (1.15±2.5 days, p=0.0002 vs. 2.3±4.5 days, p=0.0014). The authors believe that consumption of formula containing probiotics reduces the risk of the appearance of diarrhoea by a factor of 1.9 (1.33-2.6). These results show that the consumption of acidified formula containing *Bifidobacterium lactis* Bb12 has the ability to protect children against infectious diarrhoea.

Besides the effect on intestinal infections, other benefits to health from the consumption of probiotics are also being studied. A commentary from the Nutrition Committee of ESPGHAN* recognises that certain probiotic preparations offer health benefits to children. The benefits concern prevention from diarrhoea and a reduction in the severity of diarrhoea attacks. Promising results are also to be found in animal and *in vitro* studies indicating beneficial effects for the immune and digestive functions and, finally, there seems to be growing evidence of a potential ability to prevent and treat atopic eczema (8).

* ESPGHAN : European Society for Paediatric Gastroenterology Hepatology and Nutrition.

1• **Satokari RM, Vaughan EE, Favier CF (2002).** Diversity of Bifidobacterium and Lactobacillus spp. In breast-fed and formula-fed infants as assessed by 16S rDNA sequence differences. Microb Ecol Health Dis 14:97-105.

2• **Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW (2000).** Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr. 30(1):61-67.

3• **Howie PW, Forsyth JS, Ogston SA, Clark A, Florey CD (1990).** Protective effect of breast feeding against infection. BMJ. 300(6716):11-16.

4• **AFSSA (2003).** Rapport du groupe de travail "Alimentation infantile et modification de la flore intestinale".

5• **Pedone CA, Arnaud CC, Postaire ER, Bouley CF, Reinert P (2000).** Multicentric study of the effect of milk fermented by Lactobacillus casei on the incidence of diarrhoea. Int J Clin Pract. 54(9):568-71.

6• **Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH (1994).** Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344:1046-49.

7• **Chouraqui JP, Van Egroo LD, Fichot MC (2004).** Acidified milk formula supplemented with bifidobacterium lactis: impact on infant diarrhea in residential care settings. J Pediatr Gastroenterol Nutr. 38(3):288-292.

8• **Aggett PJ, Agostoni C, Axelsson I, Braegger C, Goulet O, Koletzko B, Michaelsen KF, Rigo J, Shamir R, Szajewska H, Turck D and Weaver LT (2004).** Probiotic bacteria in dietetic products for infants: A Commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 38(4):365-74.

One hypothesis that is today under debate within the scientific community highlights the involvement of indigenous flora in the pathogenesis of inflammatory bowel diseases (ulcerative colitis and Crohn's disease). This has given rise to other working hypotheses according to which the manipulation of the enteric flora by probiotics may be beneficial in treating this type of pathology. According to Fergus Shanahan from the Alimentary Pharmabiotic Centre (University College Cork, Ireland), the advantages of probiotics as a therapeutic tool resides in their harmlessness in comparison with corticosteroids and other immunosuppressive products habitually used to treat patients suffering from inflammatory bowel diseases (9).

Lactobacillus GG increases remission time in patients suffering from ulcerative colitis

Pouchitis is a result of inflammation of the surgically-installed pouch between the ileum and the anus after a colectomy. This type of operation is performed on patients suffering from recurring or fulminating ulcerative colitis in order to remove the part of the intestine affected by the disease. Such inflammatory attacks are frequent and require the administration of anti-inflammatory medicines. A Dutch team has conducted a clinical study in order to evaluate the efficacy of *Lactobacillus rhamnosus* GG against recurrence, i.e. the number of inflammatory attacks (10).

A total of 117 patients were enrolled in the study. Immediately following the ileo-recto anastomosis, 39 received fermented milk containing the probiotic ($1,4x10^{10}$ cfu/day) for 1 year and the 78 other patients made up the control group and were given no probiotics. The control patients were monitored on average for 68 months (11 to 163 months) and the test patients for 32 months (22-65 months).

During the 3-year period following the operation, fewer patients given the probiotic suffered a first attack of pouchitis than the control patients (total risk over 3 years 7 % vs. 29 % of patients, p=0.011).

This clinical study shows that the consumption of the probiotic *L. rhamnosus* GG appears to postpone the first pouchitis attack and consequently the prescription of antibiotics.

Anti-inflammatory activity of probiotics - proposal for a mechanism of action

Researchers in the United States, Japan and Israel recently performed joint research that highlighted the involvement of Toll-like receptors (TLR) in the inflammation process in murine experimental colitis (11).

TLRs are involved in the inflammatory and cytotoxic response of the macrophage by inducing TNFα, NO and IL-12 (12). Pathogenic bacteria activate the host's innate immune system via certain of its components (LPS, peptidoglycane, lipoproteins, DNA, etc.) recognized by TLRs. Five members of this receptor family, TLR 2, 4, 5, 6, 9, recognize the various components of bacterial origin.

In this study, the authors show that administering a combination of irradiated VSL#3* bacteria is as effective as a combination of live bacteria in treating murine experimental colitis. Given that the irradiated bacteria are unable to grow in culture, it is hardly likely that, *in vivo* in mice their metabolites and/or their ability to colonize the intestine could be involved in the protective effect observed. It therefore becomes possible to postulate that this anti-inflammatory activity could result from activation of the mice's innate immunity by different components of the bacteria contained in VSL#3.

The administration of VSL#3 treated with DNase, methylated VSL#3 DNA or calf thymus DNA was unable to reduce the severity of the colitis in normal mice. This indicates that the DNA of the probiotics is probably involved.

In TLR2 or TLR4 deficient mice, the severity of the colitis was strongly attenuated in the presence of VSL#3 irradiated bacteria whereas no improvement was observed when the mice used were TLR9 deficient. This result shows that it is the component of the probiotic recognized by the TLR9, to be specific the DNA, that is responsible for the anti-inflammatory activity.

The DNA of both the probiotics and the TLR9 receptors carried by the host's macrophages, appear to be the essential elements in triggering the anti-inflammatory process. This anti-inflammatory ability of the VSL#3 mixture expresses itself independently of whether the bacteria are alive or inactivated by irradiation, as what is essential is that their DNA is intact. This action mechanism explaining the anti-inflammatory ability of probiotics does not exclude other action mechanisms as yet non-elucidated.

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

9• **Shanahan F (2004).** Probiotics in inflammatory bowel disease - therapeutic rationale and role. Adv Drug Deliv Rev. 19;56(6):809-818.
10• **Gosselink MP, Schouten WR, van Lieshout LM, Hop WC, Laman JD, Ruseler-van Embden JG (2004).** Delay of the first onset of pouchitis by oral intake of the probiotic strain Lactobacillus rhamnosus GG. Dis Colon Rectum. 47(6):876-884.
11• **Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, Akira S, Takeda K, Lee J, Takabayashi K, Raz E (2004).** Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology. 126(2):520-528.
12• **Underhill DM, Ozinsky A (2002).** Toll-like receptors: key mediators of microbe detection. Curr Opin Immunol. 14(1):103-110.

Efficacy of probiotics in fighting allergic rhinitis

One field of scientific research is currently exploring the potential of probiotics to prevent the onset of allergic reactions. The working hypothesis is based on the fact that a correlation exists between the composition of the intestinal microflora and allergic reactions (13) and that consumption of probiotics modifies this composition at least temporarily. The work done by Erika Isolauri's team has shown that the probiotic *Lactobacillus rhamnosus* GG is able to prevent atopic disease in at-risk children (14, 15).

A Taiwanese team has evaluated the preventive effect of another strain of lactobacillus, *Lactobacillus paracasei-33 (LP33)* in fighting chronic allergic rhinitis (16). The clinical trial was conducted randomised, double-blind and placebo-controlled. The teenagers (14-16 year olds) enrolled in the study, had all been suffered from allergic rhinitis for at least one year. They were subjected to sensitivity tests to 5 allergens (specific IgE test), and all were found to be sensitive to dust mites. The tested group was composed of 60 patients required to consume 200 ml of fermented milk containing strains of *S. thermophilus*, *L. bulgaricus* and LP33 ($1x10^7$ cfu/ml) on a daily basis for 30 days. The control group (n=20) was given fermented milk containing only *S. thermophilus* and *L. bulgaricus*.

Changes to their symptoms and quality of life were evaluated via a standardized test*. At the end of the 30-day study, the scores obtained in this test showed an improvement in the amplitude (p=0.022) and frequency (p=0.037) of the symptoms in those patients receiving the milk containing LP33 compared to the control group. None of the patients suffered any side effects (fever, diarrhoea, etc.) after taking the fermented milk.

These results show that the consumption of fermented milk containing the probiotic *Lactobacillus paracasei-33* makes an effective contribution to improving the symptoms and discomfort caused by allergic rhinitis. Other clinical studies will however be needed before the consumption of probiotics can be envisaged as an alternative treatment for allergic rhinitis.

* PRQLQ test : Paediatric Rhinoconjunctivitis Quality of Life Questionnaire.

13• **Bjorksten B, Naaber P, Sepp E, Mikelsaar M (1999).** The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy. 29(3):342-6.
14• **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.
15• **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.
16• **Wang MF, Lin HC, Wang YY, Hsu CH (2004).** Treatment of perennial allergic rhinitis with lactic acid bacteria. Pediatr Allergy Immunol. 15(2):152-8.

Preventing allergy with probiotics: hypothesis for a mechanism of action

The ability of probiotics to reduce sensitivity to allergy may result from their ability to influence the immune response by modulating the balance between the Th1 and Th2 type responses*. A Finnish team has attempted to clarify the underlying mechanisms (17).

The goal of this research was to compare the ability of two Gram-positive bacteria - one pathogenic (*Streptococcus pyogenes*) and the other probiotic (*Lactobacillus rhamnosus* GG) - to induce maturation of dendritic cells**.

The maturation of dendritic cells is characterized by the production of chemokines and cytokines by co-stimulatory molecules and by their endocytosis potential in response to bacterial stimulation.

The tests performed *in vitro* show that *S. pyogenes* stimulates the maturation of human dendritic cells more strongly than *L. rhamnosus* GG. Stimulation by this pathogen results in a strong expression of the co-stimulatory molecules (CD80, CD83 and CD86) and the production of cytokines and chemokines characterised by a Th1 type response including IL-2 and IL-12 interleukins. Stimulation by the probiotic causes a lesser production of chemokines and cytokines, a moderate expression of the co-stimulatory molecules and the absence of IL-2 and IL-12 induction.

These results show that, *in vitro*, *S. pyogenes* and *L. rhamnosus* GG do not induce the same response in human dendritic cells. These cells are able to distinguish between pathogenic and non pathogenic Gram-positive bacteria. These different response modalities of dendritic cells result in the implementation of a suitable immune response. The probiotic and the pathogen influence the Th1 type differently in the dendritic cells, leading scientists to believe that the probiotic's ability to prevent the allergic response is based in part on a specific modulation of the equilibrium between the Th1 and Th2 responses in the dendritic cells. This conclusion is supported by the results of another team (18). This shows that the probiotic *Lactococcus lactis* G50 suppresses the Th2 response (and consequently hypersensitivity reactions) in human macrophages cultivated *in vitro*.

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

** Dendritic cells are cells specializing in antigen presentation. In their immature state, these cells are located in the peripheral tissues. Contact with an antigen triggers the maturation process that is accompanied by phenotypical and functional modifications.

17• **Veckman V, Miettinen M, Pirhonen J, Siren J, Matikainen S, Julkunen I (2004).** Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. J Leukoc Biol. 75(5):764-71.
18• **Kimoto H, Mizumachi K, Okamoto T, Kurisaki J (2004).** New Lactococcus strain with immunomodulatory activity: enhancement of Th1-type immune response. Microbiol Immunol. 48(2):75-82.

Probiotics fighting cancer - promising results in tests on mice

Yoghurt consumption inhibits cancer of the colon induced in mice by a chemical carcigenous agent. More precisely, this anti-tumour activity affects the stages of promotion and progression of the development of the tumour but does not concern the initiation stage. This ability linked to yoghurt consumption appears to be exerted through the modulation of the immune response and stimulation of the apoptotic cells (19). The cytoplasmic fraction of *Lactobacillus casei* or *Bifidobacterium longum* counters the proliferation of tumourous cells *in vitro*, and exerts an antitumourigenic effect on mice carrying tumours (20).

19• **De Moreno de Leblanc A, Perdigon G (2004).** Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. Med Sci Monit. 10(4):BR96-104.
20• **Lee JW, Shin JG, Kim EH, Kang HE, Yim IB, Kim JY, Joo HG, Woo HJ (2004).** Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of Lactobacillus casei and Bifidobacterium longum. J Vet Sci. 5(1):41-8.

Active biopeptides from lactic fermentation

A team of researchers from the Industrial Fermentation Institute (Spain) has evaluated the ability of different industrial fermented milks to inhibit the enzyme AC* (21). *In vitro*, the majority of these milks present only moderate ACE inhibition, signifying that they contain very few active peptides. Two of them, that presented significant activity, were subjected *in vitro* to enzymatic hydrolysis simulating physiological digestion. After this incubation, in particular with pancreatic juices, the anti-ACE activity was either stable or increased. The authors conclude that physiological digestion is capable of stimulating active peptide formation from the proteins present in the fermented milk.

* In humans, angiotensin plays a role in regulating arterial pressure. Angiotensin is a powerful vasoconstrictor and its vasoconstrictor effect is responsible for its hypertensive effect. The angiotensin-converting enzyme (ACE) transforms inactive angiotensin I into active angiotensin II and deactivates bradykinin. Bradykinin is an endogenous polypeptide that unlike angiotensin has a vasodilatory effect.

21• **Hernandez-Ledesma B, Amigo L, Ramos M, Recio I (2004).** Angiotensin converting enzyme inhibitory activity in commercial fermented products. Formation of peptides under simulated gastrointestinal digestion. J Agric Food Chem. 52(6):1504-10.

Colonisation of newborns with *Lactobacillus* GG - early mother-child transmission

American researchers have observed that *Lactobacillus* GG can colonize the intestine of newborn babies whose mothers consumed probiotics towards the end of pregnancy (22). Five mothers, 4 babies born by routine vaginal delivery and 1 by caesarean were involved. Lactobacillus GG was detected in the mothers' faeces at the time of birth and in the babies' faeces at the age of 1 month and 6 months. The probiotic was not administered to any of the newborn babies. According to the authors, *Lactobacillus* GG appears to have been transmitted to the babies during birth through contact with the mother's intestinal microflora, even in children born via caesarean. This study did not examine the potential benefits to health of this early colonisation.

22• **Schultz M, Gottl C, Young RJ, Iwen P, Vanderhoof JA (2004).** Administration of oral probiotic bacteria to pregnant women causes temporary infantile colonization. J Pediatr Gastroenterol Nutr. 38(3):293-7.

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www.maison-du-lait.com

23• **Asahara T, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y (2004).** Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing Escherichia coli O157:H7. Infect Immun. 72(4):2240-7.

24• **Bai AP, Ouyang Q, Zhang W, Wang CH, Li SF (2004).** Probiotics inhibit TNF-alpha-induced interleukin-8 secretion of HT29 cells.World J Gastroenterol. 10(3):455-7.

25• **Granato D, Bergonzelli GE, Pridmore RD, Marvin L, Rouvet M, Corthesy-Theulaz IE (2004).** Cell surface-associated elongation factor Tu mediates the attachment of Lactobacillus johnsonii NCC533 (La1) to human intestinal cells and mucins. Infect Immun. 72(4):2160-9.

26• **Kunz AN, Noel JM, Fairchok MP (2004).** Two cases of Lactobacillus bacteremia during probiotic treatment of short gut syndrome.J Pediatr Gastroenterol Nutr. 38(4):457-8.

27• **Lamine F, Fioramonti J, Bueno L, Nepveu F, Cauquil E, Lobysheva I, Eutamene H, Theodorou V (2004).** Nitric oxide released by Lactobacillus farciminis improves TNBS-induced colitis in rats.Scand J Gastroenterol. 39(1):37-45.

28• **Lee YK, Ho PS, Low CS, Arvilommi H, Salminen S (2004).** Permanent colonization by Lactobacillus casei is hindered by the low rate of cell division in mouse gut. Appl Environ Microbiol. 70(2):670-4.

29• **Linsalata M, Russo F, Berloco P, Caruso ML, Matteo GD, Cifone MG, Simone CD, Ierardi E, Di Leo A (2004).** The influence of Lactobacillus brevis on ornithine decarboxylase activity and polyamine profiles in Helicobacter pylori-infected gastric mucosa. Helicobacter. 9(2):165-72.

30• **Maldonado A, Jimenez-Diaz R, Ruiz-Barba JL (2004).** Induction of plantaricin production in Lactobacillus plantarum NC8 after coculture with specific gram-positive bacteria is mediated by an autoinduction mechanism. J Bacteriol. 186(5):1556-64.

31• **Montalto M, Vastola M, Marigo L, Covino M, Graziosetto R, Curigliano V, Santoro L, Cuoco L, Manna R, Gasbarrini G. Probiotic (2004).** Treatment Increases Salivary Counts of Lactobacilli: A Double-Blind, Randomized, Controlled Study. Digestion. 69(1):53-56

32• **Narva M, Nevala R, Poussa T, Korpela R (2004).** The effect of Lactobacillus helveticus fermented milk on acute changes in calcium metabolism in postmenopausal women. Eur J Nutr. 43(2):61-8.

33• **Osman N, Adawi D, Ahrne S, Jeppsson B, Molin G (2004).** Modulation of the effect of dextran sulfate sodium-induced acute colitis by the administration of different probiotic strains of Lactobacillus and Bifidobacterium. Dig Dis Sci. 49(2):320-7.

34• **Otte JM, Podolsky DK (2004).** Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. Am J Physiol Gastrointest Liver Physiol. 286(4):G613-26.

35• **Piaia M, Antoine JM, Mateos-Guardia JA, Leplingard A, Lenoir-Wijnkoop I (2003).** Assessment of the benefits of live yogurt: methods and markers for in vivo studies of the physiological effects of yogurt cultures. Microb Ecol Health Dis. 15:79-87.

36• **Saito Y, Sakamoto M, Takizawa S, Benno Y (2004).** Monitoring the cell number and viability of Lactobacillus helveticus GCL1001 in human feces by PCR methods.FEMS Microbiol Lett. 231(1):125-30.

37• **Salvini F, Granieri L, Gemmellaro L, Giovannini M (2004).** Probiotics, prebiotics and child health: where are we going?J Int Med Res. 32(2):97-108.

38• **Satokari RM, Vaughan EE, Smidt H, Saarela M, Matto J, de Vos WM (2003).** Molecular approaches for the detection and identification of bifidobacteria and lactobacilli in the human gastrointestinal tract. Syst Appl Microbiol. 26(4):572-84.

39• **Tannock GW (2004).** Can the gut microflora of infants be modified by giving probiotics to mothers?J Pediatr Gastroente-rol Nutr. 38(3):244-6.

40• **Valeur N, Engel P, Carbajal N, Connolly E, Ladefoged K (2004).** Colonization and immunomodulation by Lactobacillus reuteri ATCC 55730 in the human gastrointestinal tract. Appl Environ Microbiol. 70(2):1176-81.

41• **Xiao SD, Zhang de Z, Lu H, Jiang SH, Liu HY, Wang GS, Xu GM, Zhang ZB, Lin GJ, Wang GL (2003).** Multicenter, randomized, controlled trial of heat-killed Lactobacillus acidophilus LB in patients with chronic diarrhea. Adv Ther. 20(5):253-60.

42• **Yesovitch R, Cohen A, Szilagyi A (2004).** Failure to improve parameters of lactose maldigestion using the multiprobiotic product VSL3 in lactose maldigesters: a pilot study.Can J Gastroenterol. 18(2):83-6.

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