

# Probiotics Reference

YOGHURTS & FERMENTED MILKS

Newsletter  
of SYNDIFRAIS  
science committee

## Assessing survival of yogurt cultures to gastrointestinal transit: relevance of analytical tools



Although the positive impact of yogurt bacteria on human health has been scientifically documented, experts are still debating if *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* have to be considered as probiotics. Among the criteria for the identification and characterisation of probiotic bacteria, as defined in the most recent international guidelines, the assessment of their ability to survive after the passage through the human gastrointestinal tract is considered a key feature. Nevertheless, human gut is inhabited by a complex microbiota whose genetic heterogeneity is still far from being completely elucidated.

In recent years, molecular techniques, coupled with traditional culturing methods, allowed to perform significant advances in the investigation of the composition of the intestinal microbiota.

Studies focused on the recovery of probiotic strains from faecal samples should take into account genetic similarities between gut resident and ingested bacterial strains, in order to avoid misleading results. Therefore, the identification strategy has to be accurately designed coupling (i) a refined microbiological approach with (ii) the confirmation of the results by molecular methods. Point (i) was efficiently investigated by Mater *et al.* (2005) and Oozeer *et al.* (2006) whose use of antibiotic-insensitive starters facilitate their recovery from faecal samples. In case of studies involving commercial products, as described by Elli *et al.* (2006), the use of specifically designed selective media allowed the recognition of yogurt strains among the strong background of faecal enterococci. The resident flora can, in fact, mask the presence of yogurt bacteria on plates. These problems, together with the choice of inappropriate culturing conditions, can lead to the conclusion that yogurt cultures can not be recovered from faecal samples after ingestion, as occurred to Del Campo *et al.* (2005).

In the frame of the design of an appropriate and reliable strategy for the assessment of yogurt starters survival in the gut, molecular analytical tools can efficiently integrate microbiological methods, especially if a careful choice of the analytical strategy has been performed prior the study. The application of unreliable primer pairs in PCR, for instance, could lead to incorrect results as demonstrated by the work of Brigidi *et al.* (2003) while the support of REP-PCR and PFGE analysis allowed to Elli *et al.* (2006) to specifically recognize the administered *S. thermophilus* and *L. bulgaricus* strains among streptococci and lactobacilli present in human faeces.

These results, together with evidences from the recent scientific literature, strongly suggest that a combined approach of microbiological and molecular analysis should be applied in the assessment of the ability of yogurt culture to survive in the gut.

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### REFERENCES

- Brigidi P, Swennen E, Vitali B, Rossi M & Matteuzzi D (2003). PCR detection of *Bifidobacterium* strains and *Streptococcus thermophilus* in feces of human subjects after oral bacteriotherapy and yogurt consumption. *Int J Food Microbiol*, 81:203-209.
- Del Campo R, Bravo D, Cantón R, Ruiz-Garbajosa P, Garcia-Albiach R, Montesi-Libois A, Yuste F-J, Abraira V & Baquero F (2005). Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers. *Appl Environ Microbiol*, 71:547-549.
- Elli M, Callegari ML, Ferrari S, Bessi E, Cattivelli D, Soldi S, Morelli L, Goupil Feuillerat N & Antoine J-M (2006). Survival of yogurt bacteria in the human gut. *Appl Environ Microbiol*, 72:5113-5117.
- Mater DDG, Bretigny L, Firmesse O, Flores M-J, Mogenet A, Bresson J-L & Corthier G (2005). *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* survive gastrointestinal transit in healthy volunteers consuming yogurt. *FEMS Microbiol Lett*, 250:185-187.
- Oozeer R, Lepingard A, Mater DDG, Mogenet A, Michelin R, Seksek I, Marteau P, Doré J, Bresson J-L & Corthier G (2006). Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol*, 72:5615-5617.

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## ... An animal model for studying the functional impact of probiotics...

...on the digestive microbiota and the host



The impact of probiotics on the composition and functioning of the digestive microbiota and the interface role the microbiota may play between transiting probiotics and the host are fields that have yet to be explored.

Jeffrey I. Gordon's team have recently proposed a simplified animal model for studying the effect of probiotic species on the functional properties of gut bacterial communities [1]. The authors created the model by colonising axenic NMRI mice with *Bacteroides thetaiotaomicron*<sup>(1)</sup> (*Bt*) or *Bifidobacterium longum*<sup>(2)</sup> (*Bl*), or both at the same time. *Bt* was chosen as a model resident bacterium because it is one of the species in the dominant population of the human digestive tract. Further, its genome has been completely sequenced and it has a wide range of enzymes involved in carbohydrate hydrolysis (226 glycoside hydrolases and 15 polysaccharide lyases). *Bl*, whose genome has also been sequenced, was used in the experiments as a model probiotic bacterium.

As a first step, transcriptional profiles were obtained from RNAs extracted from the caecal content of animals colonised by one or other of the two bacteria or both. GeneChips carrying nearly 99% of the predicted genes of each genome were made. Comparison of the transcriptional profiles showed that 14.4% of the *Bt* genes present on the GeneChip<sup>(3)</sup> were differentially expressed when the bacterium was in the presence of *Bl* in the caecum (359 genes up-regulated and 322 down-regulated). Focusing the analysis on the *Bt* genes involved in degrading carbohydrates, variations in expression were found in 31 glycoside hydrolase genes and 2 polysaccharide lyase genes. Ninety-eight per cent of them were up-regulated, as for example genes coding for enzymes that degrade mannose- or xylose-containing glycans. Of the 1,722 *Bl* genes represented on the GeneChip, only 61 (3.5%) were expressed differentially when the bacterium was in the presence of *Bt* in the caecum. Of these, the genes associated with the transport and metabolism of carbohydrates appeared to be mainly down-regulated. These variations in expression of the carbohydrate degradation system when both species were present in the caecal habitat suggests changes in the nature of the growth substrates used by the bacteria. In particular, further analysis of monosaccharides available in the caecal content, combined with *in silico* metabolic simulations, seem to confirm that the presence of *Bl* expands the range of carbohydrate substrates used by *Bt*.

To extend the scope of the study to other probiotic bacteria, another species of bifidobacterium and a strain of another bacterial genus were tested in place of *Bl* in the mouse model. These strains were *Bifidobacterium animalis* DN-173 010 (*Ba*) and *Lactobacillus casei* DN-114 001 (*Lc*), both of which are used in the manufacture of commercial probiotic fermented milks. Compared to *Bl*, both *Ba* and *Lc* appear overall to have a weaker impact on the expression of *Bt*. Fifty-eight *Bt* genes were up-regulated in the presence of *Ba*, particularly genes involved in transcription and replication. The response induced by *B. animalis* thus contrasts with that of *B. longum*. *Bt* responded to *Lc* with an increased expression of 53 genes, mainly genes involved in the transport and metabolism of carbohydrates and inorganic ions. Analogous responses can therefore also be observed with members of Bacteria from different divisions.

To conclude the study, the authors used transcriptomics to analyse the response of the host's caecal epithelium to colonisation by *Bt* and/or *Bl*. Compared to the transcriptome of epithelial cells of axenic mice, few variations in gene expression was observed when the digestive tract was colonised by the micro-organisms. Of 52 genes identified as being up-regulated when both bacterial species had colonised the caecum, only 15 were also expressed at higher levels in the presence of *Bt* alone, and only 7 in the presence of *Bl* alone.

This research undoubtedly illustrates the relevance of simplified animal models for analysing the interactions between probiotics, digestive microbiota and the host. The diversity of the human microbiota and every individual's specific features should, however, lead to more complex models to improve understanding of this question in humans.

### REFERENCE

[1] Sonnenburg JL, Chen CT & Gordon JI (2006). Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biol*, 4:e413.

(1) *B. thetaiotaomicron* ATCC 29148, isolated from the faeces of a healthy adult.

(2) *B. longum* NCC2705, taken from an infant.

(3) Of 4,779 *Bt* genome sequences presumed to be coding sequences, 4,719 were placed on the chip.

In the search for probiotics to effectively treat certain inflammatory bowel diseases (IBD), it is essential to have appropriate selection tools and not rely solely on *in vitro* methods. The selected strains also need to be tested for safety, especially when the host's intestinal mucosa is damaged<sup>(1)</sup>. For these purposes, a laboratory of the Institut Pasteur in Lille (France) has developed and tested a mouse model of TNBS-induced colitis<sup>(2)</sup> [2].

Three species of lactobacillus (*L. plantarum* Lp-115, *L. salivarius* Ls-33 and *L. acidophilus* NCFM), previously selected *in vitro* for their probiotic potential and their persistence in the digestive tract, were administered to healthy mice and to mice presenting severe or very severe colitis. A non-probiotic strain of *L. paracasei* subsp *paracasei* (Lpp) isolated from the blood of a patient suffering from endocarditis was also used in the experiments. To evaluate inflammation, macroscopic scores (0 to 10) were determined using Wallace's criteria<sup>(3)</sup> and myeloperoxidase activity was measured. The safety of the strains was assessed by selectively counting the bacteria present in the mesenteric lymph nodes, spleen, liver and kidneys. The animals' weights and mortality were also monitored.

Whatever the degree of severity of the colitis, only the *L. salivarius* strain showed a significant reduction in the inflammation score. These results were in contrast with those for the Lpp strain, responsible for Osler's disease, which exacerbated the colitis and spread to the other organs.

This result illustrates the importance of conducting *in vivo* tests before making any assumptions about the properties of a potential probiotic selected *in vitro*. In all cases, it is still essential to verify whether the strain also expresses its properties in humans. The study also shows that certain strains of probiotics can translocate in conditions of extreme inflammation. While these conditions probably cannot be transposed to human subjects, the authors nevertheless recommend choosing probiotic strains whose safety profile has been assessed, especially if they are to be used for patients in a critical condition.

#### REFERENCE

[2] Daniel C, Poiret S, Goudercourt D, Dennin V, Leyer G & Pot B (2006). Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Appl Environ Microbiol*, 72:5799-5805.

(1) The damage that some IBD can cause to the intestinal mucosa increases the risk that a bacterium will pass through the mucosa (translocation), enter the blood stream and cause septicaemia.

(2) Intra-rectal administration of TNBS (2,4,6-trinitrobenzène) causes a colitis in mice that is similar to the acute colon inflammation found in patients with IBD.

(3) These criteria reflect the degree of inflammation, the thickness of the colonic mucosa and the degree of ulceration.

An increasing volume of data shows the involvement of oxygen radicals in intestinal inflammation. While superoxide dismutases (SODs) are a promising avenue for eliminating these radicals and potentially combating inflammation, a stumbling block to therapeutic use of these antioxidant enzymes is the fact that their activity quickly decreases in the body.

Scientists have been studying the possibility of using lactic acid bacteria as SOD vectors by assessing their efficacy in a model of colitis<sup>(1)</sup> in the rat [3]. Animals received four bacterial strains independently, by intragastric administration ( $10^9$  CFU per day for 4 days before and after induction of inflammation). The strains used were *Lactobacillus plantarum* (Lp) with no SOD; Lp genetically modified to overproduce an Mn-SOD (Lp-SOD+); *Lactococcus lactis* (Ll) expressing the same Mn-SOD naturally; and an Ll variant modified to overproduce the enzyme (Ll-SOD+). Unlike Lp, the three bacteria producing SOD limited the animals' weight loss, reduced the severity of the lesions at both macro- and microscopic levels and reduced myeloperoxidase activity in the colonic tissue. The SOD delivered by the bacteria thus had a significant positive effect on the inflammation, the best results being obtained with Ll-SOD+. However, these experiments could not establish any correlation between the amount of SOD produced and the intensity of the anti-inflammatory effect.

The experimental inflammation models described in the literature to date had not revealed the *in vivo* anti-inflammatory potential of SOD-producing lactic acid bacteria. While this study shows this property for the first time, it also highlights the importance of the choice of colitis model for assessing the effects of a treatment.

#### REFERENCE

[3] Han W, Mercenier A, Ait-Belgnaoui A, Pavan S, Lamine F, van Swam II, Kleerebezem M, Salvador-Cartier C, Hisbergues M, Bueno L, Theodorou V & Fioramonti J (2006). Improvement of an experimental colitis in rats by lactic acid bacteria producing superoxide dismutase. *Inflam Bowel Dis*, 12:1044-1052.

(1) Colitis induced by intracolonic infusion of TNBS

An *in vivo* tool for selecting probiotics with anti-inflammatory properties and evaluating their safety

Superoxide-dismutase-producing lactic acid bacteria to treat intestinal inflammation

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### VSL#3 stimulates mucus secretion in the rat

The probiotic cocktail VSL#3 was assessed for its effects on the protective mucus layer of the intestine of the rat [4]. After 7 days' intragastric administration of the bacterial preparation, mucus secretion was measured by evaluating incorporation of tritiated glucosamine in the luminal glycoproteins of the animals' colons. Expression of mucin genes (*muc2* in particular) in colonic cells was also quantified using amplification methods (RT-qPCR). The results show that the animals fed with the probiotic preparation produced a 60% increase in mucin secretion compared to the control animals. Expression of the *muc2* gene was also increased by a factor of 5.

For more precise observation, the authors then put colonic epithelial cells<sup>(1)</sup> in culture in contact with the mixture of bacteria that make up VSL#3 and with each of the bacteria concerned, separately. Under these conditions, no mucin secretion was observed. However, both the VSL#3 culture medium and the media from each individually cultured species caused an increase in mucin secretion. Thus the culture media of each of the four lactobacillus species<sup>(2)</sup> used in the probiotic cocktail prove as effective as the VSL#3 medium itself, while the three bifidobacterium species<sup>(3)</sup> and *Streptococcus salivarius* show less marked stimulation abilities. Paradoxically, in the conditions of the experiment mucin secretion was not accompanied by an increase in the expression of the *muc2* gene.

Additional tests were conducted to try to identify the molecular factor in VSL#3 that is responsible for inducing mucin secretion. This factor proves to be resistant to temperature (15 mins boiling), to proteases and to DNase I. The authors conclude that it could be a lipoprotein or a polysaccharide.

#### REFERENCE

[4] Caballero-Franco C, Keller K, De Simone C & Chadee K (2007). The VSL#3 probiotic formula induces mucine gene expression and secretion in colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol*, 292:315-322.

### An action mechanism of *Escherichia coli* Nissle 1917

In central Europe, *Escherichia coli* Nissle 1917 (EcN) has been used as a probiotic strain for decades. This strain can prevent diarrhoea caused by infections due to pathogens and studies suggest it is effective in the treatment of certain inflammatory diseases of the digestive tract. However, few experimental studies have looked for the cellular and molecular mechanisms underlying the beneficial effects of this probiotic.

A German team [5] investigated the molecular response of epithelial cells in culture with or without the presence of EcN and/or an enteropathogenic strain of *E. coli* (EcEP). They particularly focused on the expression and cellular localization of two proteins: ZO-2, which interacts with the membrane proteins of the tight junction and so helps to maintain the cohesion of the epithelial cells; and an isoform of protein kinase C (PKC $\zeta$ ) which, by phosphorylation, causes ZO-2 to detach from the protein complex of the tight junction, so destabilizing the epithelial barrier.

When epithelial cells were incubated in the presence of EcN, the quantity of mRNA of ZO-2 and the protein's level of expression increased. ZO-2 also concentrated at the points of contact between cells, suggesting that the protein had associated with the tight junction complex. Conversely, when cells were in the presence of EcEP, the expression of ZO-2 declined and its subcellular distribution was significantly different. At the same time, the expression, activity and localization of PKC $\zeta$  showed the opposite trends to those of ZO-2.

When epithelial cells were incubated in the presence of both EcEP and EcN, the levels of expression and the localization of the proteins studied were comparable with those in the experiments with EcN alone. The experiments thus show that the probiotic neutralizes the deleterious effects of the pathogenic strain by preventing it from destabilizing the tight junction. Additional results further show that the probiotic is capable of restoring the integrity of the epithelial barrier in cells already infected with the bacterial pathogen.

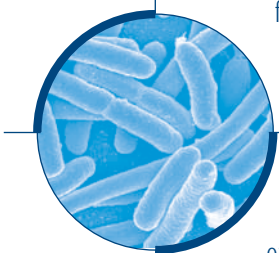
#### REFERENCE

[5] Zyrek AA, Cichon C, Helms S, Enders C, Sonnenborn U & Schmidt MA (2007). Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKC $\zeta$  redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol*, 9:804-816

(1) LS 174T cells of human adenocarcinoma constitutively expressing and secreting mucins.

(2) *L. plantarum*, *L. acidophilus*, *L. casei*, *L. delbrueckii*.

(3) *B. infantis*, *B. breve*, *B. longum*.



A clinical study was performed to study the impact of a milk fermented by *Lactobacillus acidophilus* La1 on the vitamin status of Egyptian children (average age 11 yrs) [6].

Two groups were formed, each with 12 subjects. For 6 weeks, one group (group La1) received the probiotic fermented milk ( $5 \times 10^9$  CFU per day in two doses) while the second group (control group) consumed an equivalent quantity of commercial yoghurt. Blood samples were taken, on an empty stomach, at the start and end of the protocol in order to measure concentrations of vitamin B<sub>12</sub> (*i.e.* cobalamine) and vitamin B<sub>9</sub> (*i.e.* folate) in the plasma. Two other metabolic markers were measured: methylmalonic acid (MMA), which is excreted in excess in the urine of subjects deficient in vitamin B<sub>12</sub> et B<sub>9</sub>; and total homocysteine (t-Hcy), whose concentrations in both urine and plasma increase in cases of vitamin B<sub>12</sub> deficiency.

The analysis results show that at the inclusion, one-third of the 24 study subjects were deficient in vitamin B<sub>12</sub> and one-fifth in vitamin B<sub>9</sub>. Concentrations of t-Hcy and MMA were higher than normal in half and one quarter of the subjects respectively.

In the La1 group, mean concentrations of vitamin B<sub>12</sub> and B<sub>9</sub> in the plasma increased by 52% and 56% respectively between the start and end of the protocol ( $P < 0.05$ ). The mean values observed in the control group were practically unchanged. After consuming the probiotic for 6 weeks, 1 child in 12 presented a vitamin B<sub>12</sub> deficiency and only 1 was deficient in vitamin B<sub>9</sub>. Measurements of MMA and t-Hcy in the blood or urine of subjects in group La1 also indicated a significant reduction in these two metabolites at the end of the protocol. Thus all the parameters measured indicate an improvement in the vitamin status of subjects consuming probiotic fermented milk.

The results of this study contrast with those of two earlier clinical trials which did not show any significant effects of probiotic products on vitamin status [7; 8]. While the new results are encouraging, they need to be confirmed on a larger cohort. The authors suggest several other parameters it seems to be important to take into account in this type of study, including the quantity and viability of the probiotic and the initial nutritional status of the subjects.

Be this as it may, a varied diet is the best source of B<sub>12</sub> and B<sub>9</sub> vitamins, especially for children.

#### REFERENCES

- [6] **Mohammad MA, Molloy A, Scott J & Hussein I (2006)**. Plasma cobalamin and folate and their metabolic markers methylmalonic acid and total homocysteine among Egyptian children before and after nutritional supplementation with the probiotic bacteria *Lactobacillus acidophilus* in yoghurt matrix. *Int J Food Sci Nutr*, **57**:470-480.
- [7] **Donaldson MS (2000)**. Metabolic vitamin B-12 status on a mostly raw vegan diet with follow-up using tablets, nutritional yeast or probiotic supplements. *Ann Nutr Metab*, **44**:229-234.
- [8] **Elmadfa I, Heinze C, Majchrzak D & Foissy H (2001)**. Influence of a probiotic yoghurt on the status of vitamins B1, B2 and B6 in the healthy adult human. *Ann Nutr Metab*, **45**:13-18.

There is some controversy over the possibility of treating atopic dermatitis effectively with probiotic bacteria. While some clinical trials have seemed to show a reduction in symptoms when *Lactobacillus rhamnosus* is administered to small infants, other studies have shown no effect, particularly in older children.

A new double-blind placebo-controlled clinical trial has recently been conducted [9]. For an intervention period of 8 weeks, 54 children (aged 1 to 55 months) presenting moderate to severe atopic dermatitis received milk or water containing either *L. rhamnosus* GG ( $10^{10}$  CFU per day in two doses) or a cellulose powder. Every 2 weeks, the patients were examined to monitor the evolution of their symptoms (measurement of their SCORAD score). Different specific IgE antibodies were also measured, as were several serum and faecal markers characteristic of the disease. Any use of corticosteroids and antihistamines was noted and the parents' "quality of life" was assessed by questionnaire.

Whatever the parameters measured, no significant difference could be identified between the group consuming the probiotic and the placebo group, either at the end of the study or at any time during the intervention period. These results contrast markedly with the positive effects observed in some other studies and in animal models. To explain the contradictory conclusions of the different clinical trials, the authors particularly point to differences in the degree of severity of the disease, the patients' ages and the probiotic formulation used. If it is hoped to show the possible therapeutic effects of probiotic bacteria on atopic dermatitis, future studies must endeavour to eliminate experimental biases.

#### REFERENCE

- [9] **Fölster-Holst R, Müller F, Schnopp N, Abeck D, Kreiselmaier I, Lenz T, von Rügen U, Schrezenmeier J, Christophers E & Weichenthal M (2006)**. Prospective, randomized controlled trial on *Lactobacillus rhamnosus* in infants with moderate to severe atopic dermatitis. *Brit J Dermatol*, **155**:1256-1261.

## A lactobacillus strain to make up vitamin B deficiencies

## Failure of *Lactobacillus rhamnosus* GG on atopic dermatitis

## Immunomodulation

The non-bacterial fraction (NBF) of a milk fermented by *Lactobacillus helveticus* R389 was tested for its mucosal immunomodulating potential in mouse [10]. Administration of NBF to the animals for 7 consecutive days produced a gradual and significant increase in the number of IgA-producing cells (IgA+) and in cytokine production (IL-2, IL-6, IL-10, IFN $\gamma$  and TNF $\alpha$ ). The increase in the quantities of IL-6 secreted by the small intestine epithelial cells agrees with proliferation of IgA+ cells in the lamina propria. It is known that terminal differentiation of these cells is dependent on this interleukine. The authors attribute the immunomodulating effects observed to the NBF's particular abundance of bioactive peptides produced during fermentation.

### REFERENCE

[10] Vinderola G, Matar C, Palacios J & Perdigón G (2007). Mucosal immunomodulation by the non-bacterial fraction of milk fermented by *Lactobacillus helveticus* R389. *Int J Food Microbiol*, **115**:180-186

## Pollen allergy

Three double-blind, placebo-controlled clinical trials were run to assess the effects of probiotic strains on the symptoms of Japanese cedar pollen allergy. One of the studies [11] concludes that consumption of fermented milk containing the strain *Lactobacillus casei* Shirota ( $4 \times 10^{10}$  CFU per day for 8 weeks) does not prevent allergic symptoms. The results of the other two studies [12; 13], conducted by the same laboratory at two different times, show that *Bifidobacterium longum* BB536 strain (lyophilisate absorbed with milk, quantity  $10^{11}$  CFU per day for 13 weeks) does significantly reduce allergic symptoms.

### REFERENCES

[11] Tamura M, Shikina T, Morihana T, Hayama M, Kajimoto O, Sakamoto A, Kajimoto Y, Watanabe O, Nonaka C, Shida K & Nanno M (2007). Effects of Probiotics on Allergic Rhinitis Induced by Japanese Cedar Pollen: Randomized Double-Blind, Placebo-Controlled Clinical Trial. *Int Arch Allergy Immunol*, **143**:75-82.

[12] Xiao JZ, Kondo S, Yanagisawa N, Miyaji K, Enomoto K, Sakoda T, Iwatsuki K & Enomoto T (2007). Clinical efficacy of probiotic *Bifidobacterium longum* for the treatment of symptoms of Japanese cedar pollen allergy in subjects evaluated in an environmental exposure unit. *Allergol Int*, **56**:67-75.

[13] Xiao JZ, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, Miyaji K, Iwatsuki K, Togashi H, Enomoto K & Enomoto T (2006). Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial. *Clin Exp Allergy*, **36**:1425-1435.

## Anti-inflammatory mechanism

A rat model of inflammatory bowel disease was used to study the effects of the probiotic yeast *Saccharomyces boulardii* on inflammation [14]. As well as significant improvement in the clinical and immunochemical parameters observed in the animals treated, the results suggest an action mechanism previously unknown in a probiotic [15]. The experiments show that *S. boulardii* causes T lymphocytes secreting gamma interferon (type 1 T-helper cells) to migrate into the mesenteric lymph nodes (MLN). Localised in the MLN, the lymphocytes are no longer near the site of the inflammation, where there is a consequent reduction in the production of pro-inflammatory cytokines. The phenomenon observed is thought to be due to an as-yet unidentified factor secreted by *S. boulardii*. To elucidate the mechanisms involved more precisely, this factor will have to be identified.

### REFERENCES

[14] Dalmasso G, Cottrez F, Imbert V, Lagadec P, Peyron J-F, Rampal P, Czerucka D & Groux H (2006). *Saccharomyces boulardii* inhibits inflammatory bowel disease by trapping T cells in mesenteric lymph nodes. *Gastroenterology*, **131**:1812-1825.

[15] Fiocchi C (2006). Probiotics in inflammatory bowel disease: yet another mechanism of action? *Gastroenterology*, **131**:2009-2012.

## Probiotics and cholesterol

Forty-four adults with total serum cholesterol  $\geq 4$  mmol.L<sup>-1</sup> were subjects in a 10-week clinical trial designed to assess the impact of capsules of *Lactobacillus fermentum* on their levels of low-density lipoproteins (LDL) [16]. A non-significant reduction in LDL levels was observed in the patients treated with *L. fermentum*, but also in the placebo group. Moreover, no variation was observed in total cholesterol, high-density lipoproteins (HDL) or triglycerides. Contrary to preliminary observations in animals, this study did not reveal any anticholesterolemic action by this strain of *L. fermentum* in humans.

### REFERENCE

[16] Simons LA, Amansec SG & Conway P (2006). Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. *Nutr Metab Cardiovasc Dis*, **16**:531-535.

## Perception of fermented milks in Canada

In the previous issue of *Probiotics Reference*, a study on the perception and image of fermented milks among the general population of five European countries revealed that the term "probiotic" was relatively well known in Europe: three-quarters of Germans, about half of Spanish and French people and about one-third of Poles and Danes said they had already heard of probiotic products [17]. A similar survey among Canadian students provides a glimpse of the situation in North America, where people consume less fermented milk products than in Europe [18]. The survey shows, for example, that 83% of the respondents were not familiar with the term "probiotic milk product" and 92% were unaware of any difference between conventional yogurt and other probiotic fermented milks.

### REFERENCES

[17] Syndifrais (2007). Awareness and image of probiotic products among the general public in five European countries. *Probiotics Reference*, (31):4-5

[18] Hekmat S & Koba L (2006). Fermented Dairy Products: knowledge and consumption. *Can J Diet Prac Res*, **67**:199-201.

## Research articles

[19] Assimos DG (2006). Probiotic therapy for hyperoxaluria. *Rev Urol*, **8**:170-171.

[20] Benton D, Williams C & Brown A (2007). Impact of consuming a milk drink containing a probiotic on mood and cognition. *Eur J Clin Nutr*, **61**:355-361.

[21] Briand V, Buffet P, Genty S, Lacombe K, Godineau N, Salomon J, Vandemelbrouck E, Ralaimazava P, Goujon C, Matheron S, Fontanet A & Bouchaud O (2006). Absence of efficacy of nonviable *Lactobacillus acidophilus* for the prevention of traveler's diarrhea: a randomized, double-blind, controlled study. *Clin Infect Dis*, **43**:1170-1175.

[22] Bu HF, Wang X, Zhu YQ, Williams RY, Hsueh W, Zheng X, Rozenfeld RA, Zuo XL & Tan XD (2006). Lysozyme-modified probiotic components protect rats against polymicrobial sepsis: role of macrophages and cathelicidin-related innate immunity. *J Immunol*, **177**:8767-8776.

[23] Canchaya C, Claesson MJ, Fitzgerald GF, van Sinderen D & O'Toole PW (2006). Diversity of the genus *Lactobacillus* revealed by comparative genomics of five species. *Microbiology*, **152**:3185-3196.

[24] Cinque B, Di Marzio L, Della Riccia DN, Bizzini F, Giuliani M, Fanini D, De Simone C & Cifone MG (2006). Effect of *Bifidobacterium infantis* on Interferon-gamma-induced keratinocyte apoptosis: a potential therapeutic approach to skin immune abnormalities. *Int J Immunopathol Pharmacol*, **19**:775-786.

[25] De Preter V, Vanhoutte T, Huys G, Swings J, De Vuyst L, Rutgeerts P & Verbeke K (2007). Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *Am J Physiol Gastrointest Liver Physiol*, **292**:G358-368.

[26] Fujimura S, Kato S, Oda M, Miyahara M, Ito Y, Kimura K, Kawamura T, Ohnuma M, Tatenno H & Watanabe A (2006). Detection of *Lactobacillus gasseri* OLL2716 strain administered with yogurt drink in gastric mucus layer in humans. *Lett Appl Microbiol*, **43**:578-581.

[27] Geier MS, Butler RN, Giffard PM & Howarth GS (2007). *Lactobacillus fermentum* BR11, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulfate sodium (DSS) in rats. *Int J Food Microbiol*, **114**:267-274.

[28] Gratz S, Taubel M, Juvonen RO, Viluksela M, Turner PC, Mykkanen H & El-Nezami H (2006). *Lactobacillus rhamnosus* strain GG modulates intestinal absorption, fecal excretion, and toxicity of aflatoxin B(1) in rats. *Appl Environ Microbiol*, **72**:7398-7400.

[29] Gueniche A, Benyacoub J, Buetler IM, Smola H & Blum S (2006). Supplementation with oral probiotic bacteria maintains cutaneous immune homeostasis after UV exposure. *Eur J Dermatol*, **16**:511-517.

[30] Hammerman C, Bin-Nun A & Kaplan M (2006). Safety of probiotics: comparison of two popular strains. *Bmj*, **333**:1006-1008.

[31] Hirose Y, Murosaki S, Yamamoto Y, Yoshikai Y & Tsuru T (2006). Daily intake of heat-killed *Lactobacillus plantarum* L-137 augments acquired immunity in healthy adults. *J Nutr*, **136**:3069-3073.

[32] Kim Y, Han KS, Imm JY, Oh S, You S, Park S & Kim SH (2006). Inhibitory effects of *Lactobacillus acidophilus* lysates on the cytotoxic activity of shiga-like toxin 2 produced from *Escherichia coli* O157:H7. *Lett Appl Microbiol*, **43**:502-507.

[33] Lahtinen SJ, Gueimonde M, Ouwehand AC, Reinikainen JP & Salminen SJ (2006). Comparison of four methods to enumerate probiotic bifidobacteria in a fermented food product. *Food Microbiol*, **23**:571-577.

[34] Lionetti E, Miniello VL, Castellaneta SP, Magista AM, de Canio A, Maurogiovanni G, Ierardi E, Cavallo L & Francavilla R (2006). *Lactobacillus reuteri* therapy to reduce side-effects during anti-*Helicobacter pylori* treatment in children: a randomized placebo controlled trial. *Aliment Pharmacol Ther*, **24**:1461-1468.

[35] Ljungberg M, Korpela R, Ilonen J, Ludvigsson J & Vaarala O (2006). Probiotics for the prevention of beta cell autoimmunity in children at genetic risk of type 1 diabetes—the PRODIA study. *Ann N Y Acad Sci*, **1079**:360-364.

[36] Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, et al. (2006). Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci U S A*, **103**:15611-15616.

[37] Massi M, Ioan P, Budriesi R, Chiarini A, Vitali B, Lammers KM, Gionchetti P, Campieri M, Lembo A & Brigidi P (2006). Effects of probiotic bacteria on gastrointestinal motility in guinea-pig isolated tissue. *World J Gastroenterol*, **12**:5987-5994.

[38] McLaughlin SD, Clark SK, Nicholls RJ, Tekkis PP & Ciclitira PJ (2006). Effective probiotic treatment is rarely cheap. *Bmj*, **333**:1272.

[39] Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, Radke M & Blaut M (2006). Effects of *Bifidobacterium lactis* Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *J Clin Microbiol*, **44**:4025-4031.

[40] Morita H, He F, Kawase M, Kubota A, Hiramatsu M, Kurisaki J & Salminen S (2006). Preliminary human study for possible alteration of serum immunoglobulin E production in perennial allergic rhinitis with fermented milk prepared with *Lactobacillus gasseri* TMC0356. *Microbiol Immunol*, **50**:701-706.

[41] Nemeth E, Fajdiga S, Malago J, Koninkx J, Tooten P & van Dijk J (2006). Inhibition of Salmonella-induced IL-8 synthesis and expression of Hsp70 in enterocyte-like Caco-2 cells after exposure to non-starter lactobacilli. *Int J Food Microbiol*, **112**:266-274.

[42] Nguyen TD, Kang JH & Lee MS (2007). Characterization of *Lactobacillus plantarum* PH04, a potential probiotic bacterium with cholesterol-lowering effects. *Int J Food Microbiol*, **113**:358-361.

[43] Olivares M, Paz Diaz-Ropero M, Gomez N, Sierra S, Lara-Villoslada F, Martin R, Miguel Rodriguez J & Xaus J (2006). Dietary deprivation of fermented foods causes a fall in innate immune response. Lactic acid bacteria can counteract the immunological effect of this deprivation. *J Dairy Res*, **73**:492-498.

[44] Rokka S, Pihlanto A, Korhonen H & Joutsjoki V (2006). In vitro growth inhibition

of *Helicobacter pylori* by lactobacilli belonging to the *Lactobacillus plantarum* group. *Lett Appl Microbiol*, **43**:508-513.

[45] Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M & Okumura K (2006). Interleukin-12 is involved in the enhancement of human natural killer cell activity by *Lactobacillus casei* Shirota. *Clin Exp Immunol*, **146**:109-115.

[46] Taylor A, Hale J, Wiltschut J, Lehmann H, Dunstan JA & Prescott SL (2006). Evaluation of the effects of probiotic supplementation from the neonatal period on innate immune development in infancy. *Clin Exp Allergy*, **36**:1218-1226.

[47] Taylor AL, Hale J, Wiltschut J, Lehmann H, Dunstan JA & Prescott SL (2006). Effects of probiotic supplementation for the first 6 months of life on allergen- and vaccine-specific immune responses. *Clin Exp Allergy*, **36**:1227-1235.

[48] Wang Z, Xiao G, Yao Y, Guo S, Lu K & Sheng Z (2006). The role of bifidobacteria in gut barrier function after thermal injury in rats. *J Trauma*, **61**:650-657.

[49] Wong C & Ustunol Z (2006). Mode of inactivation of probiotic bacteria affects interleukin 6 and interleukin 8 production in human intestinal epithelial-like Caco-2 cells. *J Food Prot*, **69**:2285-2288.

[50] Yadav H, Jain S & Sinha PR (2006). Effect of skim milk and dahi (yogurt) on blood glucose, insulin, and lipid profile in rats fed with high fructose diet. *J Med Food*, **9**:328-335.

## Review articles

[51] Donohue DC (2006). Safety of probiotics. *Asia Pac J Clin Nutr*, **15**:563-569.

[52] Ewaschuk JB & Dieleman LA (2006). Probiotics and prebiotics in chronic inflammatory bowel diseases. *World J Gastroenterol*, **12**:5941-5950.

[53] Ewaschuk JB, Tejpar QZ, Soo I, Madsen K & Fedorak RN (2006). The role of antibiotic and probiotic therapies in current and future management of inflammatory bowel disease. *Curr Gastroenterol Rep*, **8**:486-498.

[54] Geier MS, Butler RN & Howarth GS (2006). Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer? *Cancer Biol Ther*, **5**:1265-1269.

[55] Lewis G (2006). Probiotics: a better way to treat infections during pregnancy. *Midwifery Today Int Midwife*, **(79)**:30-31.

[56] Marteau P (2006). Probiotics, prebiotics, synbiotics: ecological treatment for inflammatory bowel disease? *Gut*, **55**:1692-1693.

[57] Michail S, Sylvester F, Fuchs G & Issenman R (2006). Clinical efficacy of probiotics: review of the evidence with focus on children. *J Pediatr Gastroenterol Nutr*, **43**:550-557.

[58] Rolfe VE, Fortun PJ, Hawkey CJ & Bath-Hextall F (2006). Probiotics for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev*, **(4)**:CD004826.

[59] Sanders ME (2006). Summary of probiotic activities of *Bifidobacterium lactis* HN019. *J Clin Gastroenterol*, **40**:776-783.

[60] Yan F & Polk DB (2006). Probiotics as functional food in the treatment of diarrhea. *Curr Opin Clin Nutr Metab Care*, **9**:717-721.

# Probiotics Reference

YOGHURTS & FERMENTED MILKS

## ... THE SCIENCE COMMITTEE

### *Its mission*

- The Syndifrais science committee brings together scientists and researchers from universities and from the fresh milk products industry.
- It is an independent body which seeks out and publicises scientific evidence of the health benefits and harmlessness of the live lactic acid bacteria in yoghurt and fermented milks.
- One of its tasks is to make a critical scan of the literature on the physiological action of probiotics and the mechanisms of such action. The results of this science survey are published in the newsletter Probiotics Reference.

### *The members*

- **Pr. Jean-Louis Bresson, Chairman** - Hôpital Necker, Paris
- **Dr. Denis Mater, Coordinator** - Syndifrais
- **Dr. Nadine Cerf-Bensoussan** - Hôpital Necker, Paris
- **Dr. Jean Fioramonti** - French National Institute of Agronomy Research (INRA)
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- **Dr. André Ayerbe** - Arilait Recherche
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- **Dr. Purification Relano** - Danone Research
- **Mrs. Brigitte Rousseau** - Yoplait

## Spotlight on



### **Probiotics, Prebiotics & New Foods**

The 4th Probiotics, Prebiotics & New Foods Congress will be held in Rome (Italy) at the Università Urbaniana. This year the Congress will be held jointly with the 30th International Congress on Microbial Ecology and Disease organised by the SOMED (Society of Microbial Ecology and Disease).

The joint meeting will start on September 16th in the early afternoon and will end on September 18th around noon. On September 18th, registered participants will also be able to participate to the 3rd YLFA-International workshop on Probiotic Fermented Milks and Public Health.

The scientific programme will feature more than 100 individual

colloquia, symposia, roundtable discussions, award lectures, oral papers and poster sessions. The Scientific Committees have endeavoured to create a well-rounded, up-to-date programme. This year's schedule will begin at 2:30 p.m. on Sunday, September 16th, with a Consensus Conference on Prebiotics organized by the FAO, followed by guest lectures.

For more information and the scientific programme in detail:  
<http://www.probiotics-prebiotics-newfood.org/>  
For details on participation in the 3rd YLFA-International workshop:  
<http://www.emeeetingeconsulting.com/ylfa/>

## ... Newsletter of the Syndifrais science committee

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