Investigating the effects of probiotics on *Helicobacter pylori* infections

Prevention of atopic disease by *Lactobacillus GG*

Efficiency of probiotics on urogenital infections

Lowering the incidence of diarrhea by probiotic

Improving lactose digestion by yoghurt

Enhancement of Natural Killer activity

Tumorigenesis and chemopreventive role of *L. casei Shirota*

Identification of intestinal microflora

Fate of the probiotics in the intestine

Lactic acid bacteria viability or implantation in the digestive tract: two unmistakable features

Robert Ducluzeau

At present is commonly accepted that efficient lactic acid bacteria used as probiotics have to be alive in the gastrointestinal tract. However does this mean that lactic acid bacteria must be implanted in vivo in the gastrointestinal tract or do the bacteria just must survive during the transit time?

Today it is demonstrated that a probiotic never becomes implanted. Fortunately this is better. The microbiological and physiological barriers exerted by the digestive tract and the autochthonous flora, towards environmental microorganisms, participate to the homeostasis and allow the host the possibility to adapt to the often unfriendly environmental modifications. However, in order to exert their beneficial effects in the host, bacteria have to be alive during their transit time. Recent works using molecular tools and designed for *Streptococcus thermophilus* clearly demonstrated that bacteria do not multiply in vivo but they still able to hydrolyse lactose all along the intestine.

In other respects, as reported by different authors, bacterial population must account between $5\times10^6$ and $10^8$ of living bacteria per gram of intestinal content in order to produce enough active molecules capable to modify the health. Nevertheless, bacteria strains are not equal towards their ability to resist to unfriendly conditions encountered in the digestive tract. Some populations are drastically reduced during their transit in the intestine and others contrarily resist. In the last case, the approximately $10^8$ living cells ingested in 100 ml of fermented milk lead to local concentrations of $10^8$ per gram of intestinal content even they are diluted 1000 fold. Thus, effects on the host may be observed.

For the moment, we just can sort out the strains that show the higher viability during the transit in the gastrointestinal tract. And, little is know about the physiological criteria leading to this viability.

We can thus say that the investigation of the characteristics, of lactic acid bacteria used as probiotics, responsible for the definitive implantation in the digestive tract is a useless approach, which can be hazardous in the worse case, if it succeeds. Contrarily, the investigation of characteristics favourable to the viability of the strains during the transit certainly deserves new developments, authorized by the progress of our knowledge on the molecular biology of lactic acid bacteria.
Investigating the effects of probiotics on *Helicobacter pylori* infections

The gastric pathogen *Helicobacter pylori* is the principal cause of peptic ulcers and the major risk factor for gastric cancers in humans. Resolution of *H. pylori*-associated disease by oral administration of antimicrobial agents is not always successful, and may be associated with adverse effects like bloating, diarrhoea and taste disturbance. Recent reports document a protective role for exogenous lactobacilli in preventing and minimizing these side effects, or in suppressing infection in humans.

A pilot study conducted on 120 healthy asymptomatic adults screened positive for *H. pylori* describes the effects of the oral administration of Lactobacillus GG during and after anti-*H. pylori* standard triple therapy (454). The treated group received the same therapy than the control and a daily supplement of 12x10^9 live Lactobacillus GG during and a week after eradication therapy. The results show no significant differences between the groups with respect to success in *H. pylori* eradication. However, the intake of Lactobacillus GG has a beneficial effect on the high incidence of gastrointestinal side effects observed in the control group and on overall treatment tolerance.

Another team from the Meiji Milk Products examined the efficacy of yoghurt supplemented with Lactobacillus gasseri against *H. pylori* in 31 infected individuals (518). Compared to the yoghurt-control group, the daily intake of the *L. gasseri*, during an 8 weeks period, seems to be effective in both suppressing *H. pylori* and reducing gastric mucosal inflammation.

The aim of this third in vivo study, designed for 53 *H. pylori*-infected volunteers, is to investigate the effect of *L. johnsonii* La1 (LC1, Nestlé strain) associated with antibiotic on *H. pylori* density, gastric inflammation and activity (463). The study followed a randomised, double-blind, and placebo-controlled design. The subjects received either the LC1-fermented milk or the placebo for three weeks, and during the two last weeks volunteers in both groups were treated with antibiotic. It was observed that LC1 ingestion induced a decrease in *H. pylori* density in the antrum and in the corpus of the stomach, and also reduced the inflammation. This effect persists for several weeks after stopping LC1 treatment.

The mode of action of Lactobacillus on *H. pylori* is not yet determined. According to in vitro results obtained on 17 strains of Lactobacillus and 10 strains of *H. pylori*, it seems that the different antagonistic effects of lactobacilli spent broth upon *H. pylori*, are related to lactobacilli acid production and to proteinaceous compounds released after lactobacilli lysis (471). In conclusion, in the in vivo studies demonstrate that probiotic supplementation has a beneficial effect on *H. pylori* infections in humans. However, double-blind placebo randomised trials remain essential to confirm these results and further studies are needed to evaluate whether these effects are maintained during long-term probiotic ingestion as well as to determine the most efficient strain of probiotic.

Prevention of atopic disease by *Lactobacillus GG*

In the issue of April 2001 of The Lancet, a double-blind placebo controlled study conducted by a Finnish team shows that perinatal administration of *Lactobacillus GG* halved the subsequent occurrence of atopic diseases in at-risk infants (513). The 159 families recruited for the study have a family history of atopic disease i.e. eczema, allergic rhinitis or asthma. The mothers received capsules of Lactobacillus GG or placebo daily during two weeks before expected delivery. After delivery, breastfeeding mothers could not take the capsules. Otherwise babies received orally the probiotic. Both these modes of administration have resulted in similar amounts of *Lactobacillus GG* in infants’ faeces. The infants were examined during 2 years, the outcome measure was atopic disease. Of the 132 children aged 2 years, atopic eczema was diagnosed in 46, asthma in six and allergic rhinitis in one. The frequency of atopic eczema, which is the main sign of atopic disease in the first years of life, in the probiotic group was half that of the placebo group (22% vs. 46%). This study shows first that Lactobacillus GG was effective in prevention of early atopic disease in children at high risk and secondly the success of this immunotherapy is dependent on the timing of probiotic administration. The exact mechanisms by which probiotics may affect atopic disease and may act as an immunomodulator remain speculative. However, there is increasing evidence that specific input from the intestinal flora to the innate immune system is essential for the establishment and maintenance of mucosal immune tolerance (Murch 2000 & 2001).

Improving lactose digestion

It is well established that persons with lactose maldigestion experience between intake and tolerance of the lactose contained in the yoghurt than in milk that contained in the yoghurt than in milk (see for review 500 and 501). Two mechanisms may explain the improved digestive tolerance observed with yoghurt: 1) digesta of the lactose is improved by the lactobacilli present in the yoghurts and/or 2) the transit time of yoghurt is higher than of the milk. A double-blind design in which lactose malabsorbers received for 5 days fresh yoghurt (containing Lactobacillus acidophilus OLL2716) and then fresh milk showed no difference in lactose digestion and tolerance during the two last weeks volunteers in both groups were treated with antibiotic. It was observed that LC1 ingestion induced a decrease in *H. pylori* density in the antrum and in the corpus of the stomach, and also reduced the inflammation. This effect persists for several weeks after stopping LC1 treatment.

Reference:


Efficiency of probiotics against *Helicobacter pylori* infection

Urogenital infections are very common with up to 300 million cases worldwide per year (502). They are generally resolved by antibiotic therapy, however, drug resistance to commonly used antibiotics and recurrence of infections remains a problem (503). The use of probiotics in the area of anti-infection provide a reliable alternative treatment and preventive regimen to antibiotics which is an up to date question. Reid and collaborators reported the clinical evidence that Lactobacillus and/or *H. pylori* can be delivered to the vagina following oral administration. It is well established that persons with lactose maldigestion experience between intake and tolerance of the lactose contained in the yoghurt than in milk (see for review 500 and 501). Two mechanisms may explain the improved digestive tolerance observed with yoghurt: 1) digesta of the lactose is improved by the lactobacilli present in the yoghurts and/or 2) the transit time of yoghurt is higher than of the milk. A double-blind design in which lactose malabsorbers received for 5 days fresh yoghurt (containing Lactobacillus acidophilus OLL2716) and then fresh milk showed no difference in lactose digestion and tolerance during the two last weeks volunteers in both groups were treated with antibiotic. It was observed that LC1 ingestion induced a decrease in *H. pylori* density in the antrum and in the corpus of the stomach, and also reduced the inflammation. This effect persists for several weeks after stopping LC1 treatment.

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Efficiency of probiotics on urogenital infections

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Lowering the incidence of diarrhoea by probiotic

A study conducted by researchers of Danone group aimed to determine if supplementation of healthy children by Actimel®, a milk fermented by yoghurt starters and Lactobacillus casei strain DN-114001 (478). This study was a multicentre, randomised, and double-blind trial, conducted over 4 months, on 928 children aged 6-24 months. Subjects were supplemented daily by standard yoghurt or by Actimel®. It was observed a statistically significant difference between the groups, the incidence of diarrhoea being reduced by Actimel® intake (15.9%) compared with yoghurt (22%). These findings suggest that L. casei provides an additional benefit in acute diarrhoea compared to standard yoghurt.

Improving lactose digestion by yoghurt

It is well established that persons with lactose maldigestion experience better digestion and tolerance of the lactose contained in the yoghurt than of that contained in milk (see for review 500 and 501). Two mechanisms may explain the improved digestibility observed with yoghurt: 1) digestion of the lactose is improved by the lactase contained in the yoghurt bacteria and/or 2) the transit time of yoghurt is higher than of the milk. A double-blind design in which 22 lactose malabsorbers received for 15 days fresh yoghurt (containing living bacteria) or heated yoghurt (followed by a cross-over after a wash-out period), the gastric emptying and the transit are assessed (515). The authors show that compared to heated yoghurt, the consumption of fresh yoghurt, which oraocecal transit time is longer, induces lower severity of gastrointestinal symptoms that is inversely correlated with orocecal transit time. The conclusion is that lactose digestion and tolerance of fresh yoghurt is improved in comparison to heated yoghurt and this effect should be mainly attributed to the presence of living bacteria.

The American Journal of Clinical Nutrition published in February 2001 a supplement “Probiotics & Prebiotics” related to the proceedings of a symposium held in Kiel, Germany - June 11-12,1998
Immunostimulatory effect of Lactic acid bacteria

Immunostimulatory effect of yoghurt and fermented milks has been proposed and investigated in animals and humans. In the following presented publications, the authors measure the activity of Natural Killer (NK) cells that are a leucocyte type. It is known that NK cells carry out the active killing of virus infected or tumorigenic cells. The activity of NK cells is measured by cytotoxic assays.

Enhancement of Natural Killer activity

A study designed with the strain *Lactobacillus casei* Shirota from Yakult Co. Ltd consisted of 9 adults who were healthy but had relatively low levels of NK activity (474). Every day during 3 weeks, half of them drank one bottle of Yakult (4x10^10^ of live bacteria), the others drank the same milk but without bacteria. Compared with the value before intake, NK cell activity is increased since one week after the start of intake of the fermented milk drink. Two months after, the activity was found to have returned to almost the same level as that before beginning the experiment. In the control group, NK cell activity did not significantly change at any time of the experiment.

Thus, it seems that the continuous intake of the fermented milk with *L. casei* Shirota enhances NK cell activity in humans and consequently may strengthen the response to infectious diseases.

An other trial, which enrolled 50 healthy adults, leads to the same conclusion (461). It was demonstrated that the consumption of non-fermented milk containing *Bifidobacterium lactis* HN019 induced the increase of polymorphonuclear cell phagocytosis and NK activity.

The authors suggest the possibility that probiotics may be used for improving NK activity in persons who usually have low levels of NK activity like elderly persons or patients with autoimmune disease or cancer.

Tumorigenesis and chemopreventive role of *L. casei* Shirota

NK cells are among the candidates for cells producing direct tumour cell destruction and may the first line of host defence against tumorigenesis in humans. A study conducted by the Yakult Central Institute for Microbiological Research aimed to evaluate the effect of *Lactobacillus casei* Shirota on tumour onset and the involvement of NK cells (520). They used two kinds of mice: normal mice (C3H/HeN) and “Beige” mice that is genetically deficient in NK cells. Carcinogenesis is chemically induced. The control mice are injected intradermally with the carcinogen and the experimental mice were fed with *L. casei* Shirota from the day of carcinogen injection onward.

In normal mice, tumour incidence in probiotic fed mice was 0 at week 6 and 42% at week 11 as opposed to 33% and 83% respectively for control mice. It seems that the probiotic delayed tumour onset and reduced tumour incidence in normal mice. Additionally, it was observed that in the experimental group, the splenic activity of NK cells is increased and this is mainly depended on the increased proportion of NK cells. In “Beige” mice, *L. casei* Shirota failed to suppress the tumorigenesis.

These findings suggest that the intake of *L. casei* Shirota delays the development of chemically induced tumour in mice and NK cells are involved in this process. The detailed nature in which the probiotic works is not known, we have to explain how the ingested probiotic, and thus located in the intestines, induces a systemic effect in the host.


In order to provide information on the effect of a probiotic on indigenous strains inhabiting the intestinal tract of humans and animals, it is crucial to dispose of methods that could be used to analyse the composition of bacterial population before, during and after the administration of the probiotic. Differentiation of isolates into species is crucial and can be difficult because of the considerable variation in biochemical attributes like fermentation profiles that seem occur among strains currently considered to represent the same species. Today taxonomical methods are based on phenotypic characterisation and genotypic analysis. The rapid and reliable identification of bacterial isolates is now greatly aided by the availability of Polymerase Chain Reaction (PCR) and automated nucleotides base sequencing of amplified DNA (499). However, the PCR-based and oligonucleotides probe methods cannot differentiate between strains belonging to the same bacterial species. These can be achieved by the use of molecular typing methods as summarised in the following table.

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilocus enzyme electrophoresis</td>
<td>Characterisation of isolates by the relative electrophoretic mobilities of a large number of enzymes</td>
</tr>
<tr>
<td>Pulsed-Field gel electrophoresis (PFGE)</td>
<td>Characterisation of isolates based on restriction fragment length polymorphisms of chromosomal DNA</td>
</tr>
<tr>
<td>Ribotyping</td>
<td>Bacterial DNA is digested and probed with ribosomal RNA gene sequence. The patterns produced provide a means of differentiating bacterial strains.</td>
</tr>
<tr>
<td>Random amplification of polymorphic DNA (RAPD)</td>
<td>A short primer is arbitrarily selected and allowed to anneal bacterial DNA. The primers hybridise at random sites to initiate DNA polymerisation in the PCR. The proximity, number and location of these priming sites vary between strains and the electrophoretic pattern of the amplified DNA fragments provides a fingerprint characteristic of each bacterial strain.</td>
</tr>
</tbody>
</table>

Unfortunately, the methods presented in the table are expensive, time consuming and generally not adapted to day-to-day analysis. A team from the Institut Pasteur developed recently a method based on PCR amplification of 16S RNA coding fragment taken advantage of the high degree of the high variability of the V1 region (460). This method was shown to work on single colonies and its validation was performed by applying it to six Lactobacillus reference strains and to various species of bacteria.

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<tr>
<td>Rapid PCR-based procedure to identify lactobacilli - application to six common Lactobacillus species. (499)</td>
<td>A DNA sequence of the 16S rRNA gene was amplified by PCR using primers specific for Lactobacillus and used as targets for hybridisation with a set of probes or restriction endonucleases.</td>
</tr>
<tr>
<td>Molecular assessment of intestinal microflora</td>
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Fate of the probiotics in the intestine

It is generally agreed that the assessment of potential probiotics involves assessment of viability of the probiotic in the host’s gut, what it means that before reaching the intestinal tract, probiotic must first survive transit through the stomach. Although it is believed that the maximum probiotic effect can be achieved if the organisms adhere to intestinal mucosal cells as Dunne et al. (2001) reported in their review (496), there is no evidence that exogenously administered probiotics do adhere to the mucosal cells in vivo. In vitro studies showed that probiotics are able to attach to cultured cells like CaCo2 and HT29 cells or to intestinal mucus extracted from faeces or to glycoproteins extracted from faecal mucus. However is this true in vivo? As Beckovorainy reports in a recent review (498) the established feature is that the probiotics are unable to become implanted in the gastrointestinal tract of the host. In fact, when the administration of probiotic strain is stopped it was no longer recovered in faeces, thus it seems that the probiotic is not able to colonize the host. Colonization may be unnecessary to achieve positive results induced by probiotic intake. The important criterion is that the probiotic continue to be metabolically active in the gut, thus providing health benefits to the host. And to obtain a continuous probiotic effect, the probiotic culture must be ingested continually.


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

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IN THIS ISSUE

- Investigating the effects of probiotics on Helicobacter pylori infections
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- Efficiency of probiotics on urogenital infections
- Lowering the incidence of diarrhoea by yoghurt
- Enhancement of Natural Killer activity
- Tumorigenesis and chemopreventive role of L. casei Shirota
- Identification of intestinal microflora
- Fate of the probiotics in the intestine