Among the main important characteristics of a good probiotic, it is usually mentioned that attachment to intestinal mucosa is an important feature. However this concept refers in fact to two distinct properties: the adhesion to membranes and the attachment to mucus.

Several strains of bacteria hold on their cell wall molecular structures able to recognize complementary structures on epithelial cells membranes and thus to attach to the intestinal mucosa.

Other bacteria have chemotropism for the intestinal mucus within carbohydrates represent their preferred source of energy: consequently these strains grow in places where the mucus is the more abundant what it means near the mucosa. What it is the advantage for a probiotic to express one or another of the attachment properties? According to an old and very frequent idea, probiotic must attach to mucosa with the aim to persist or to become implanted in the intestinal tract. However, today it is widely demonstrated that probiotics, which are active during the transit along the digestive tract, do never become implanted. In other respects, most of the bacteria from the indigenous flora don’t adhere to intestinal mucosa. The attachment of a strain is not a necessary condition for persistence or in vivo activity of the probiotic.

Since a long time, it was known that pathogenic bacteria, like several strains of Escherichia coli bear attachment factors that allow them to resist to peristaltic effects and thus to develop in the small intestine. Thus it emerged the hypothesis that it may be possible to saturate the attachment sites with non-pathogenic microorganisms in place of the pathogenic ones. However, this has never been demonstrated in vivo. Attachment sites are very specific, consequently how lactic acid bacteria may saturate the attachment sites of a Gram-negative pathogenic bacteria? Additionally, the quick replacement of the mucosa induces new sites of attachment that are not saturated.

Finally, since the immunostimulating role of probiotics was investigated another hypothesis emerged: the stimulation must be strongest if bacteria are attached to mucosa. This has never been demonstrated. Contrarily, it can be hypothesised that a continuous stimulation by bacterial antigens may be deleterious.

At last, and in opposition to the current opinion, there is no reason to think that the attachment of probiotics to the intestinal mucosa must be a useful feature, and the possibility that this property may be dangerous is questionable.
Alleviating infectious diarrhoea in children: a promise of probiotics

Diarrhoea is a major paediatric health problem worldwide. The predominant pathogens implicated in infectious diarrheal disease in neonates are rotavirus and Escherichia coli. The rationale for using probiotics in infectious diarrhoea is based on the assumption that they act against intestinal pathogens. In fact, certain lactic acid bacteria strains have been reported to reduce duration, severity, incidence or a combination thereof of infant of infant diarrhoea in some cases. A review (1) tried to assess and to quantify the evidence from published, randomised, controlled trials on the effectiveness of probiotics in the treatment and prevention of acute infectious diarrhoea in infants and children (1 to 48 months). This review excluded trials without randomisation and comparative studies without placebo. A total of 13 papers met the inclusion criteria. The probiotic strains studied were L. GG, L. reuteri, L. acidophilus LB, Saccharomyces boulardii, Streptococcus thermophilus and L. bulgaricus.

The authors concluded that there is an evidence of a clinically significant benefit of probiotics in the treatment of acute infectious diarrhoea in infants and children, particularly in rotavirus gastroenteritis. Lactobacillus GG showed the most consistent effect, although other probiotic strains may also be effective.

...which it is not confirmed in adults antibiotic-associated diarrhoea

Diarrhoea is also a well-known complication of antibiotic therapy. Estimates of the risk of antibiotic-associated diarrhoea vary between 5% and 30% depending on the antibiotic administered (2).

To assess the efficacy of Lactobacillus GG in preventing antibiotic-associated diarrhoea in adults, a team of the Mayo Clinic conducted a randomised, double-blind, placebo controlled trial with 267 hospitalised adults (2). These patients receiving antibiotics were randomised to receive L. GG 20x10^9 cfu/day or placebo for 14 days. Diarrhoea developed in 29.3% of patients of the L. GG group and in 29.9% in the patients receiving the placebo (p=0.93). It seems that L. GG was not efficient in the dose used to reduce the rate of occurrence of diarrhoea in adult patients taking antibiotics since diarrhoea developed in 29.3% of patients of the L. GG group and in 29.9% in the patients receiving the placebo. While showing promise in alleviating infectious diarrhoea in children, LGG may not be effective in adults taking antibiotics. Potential explanations for this difference include differences in the aetiology of the diarrhoea and differences in the gastrointestinal microflora between adults and children.

Since now, this scientific letter "Yoghurts & fermented milks" is also available on the website of La Maison du Lait:

www.maison-du-lait.com
The mechanisms through which L. GG antagonizes pathogenic gastrointestinal organisms and exerts beneficial effects in the host are poorly understood. Because nitric oxide is a local mediator, which regulates several physiological, and pathophysiological processes in human body and seems to be involved in intestinal mucosa protection (3, 4), a team from the University of Tampere in Finland investigated if nitric oxide synthesis is induced in response to L. GG (5). These researchers showed that in cultured macrophages and human T84 intestinal epithelial cells cultured L. GG induces nitric oxide production through induction of nitric oxide synthase by a mechanism involving activation of transcription factor NF-κB*.

The induction of low-level production of nitric oxide by L. GG is supposed to function as a protective mechanism in the intestinal mucosa and to regulate immune cell functions. In addition, nitric oxide has antimicrobial effects and acts as a microbial killing mechanism in activated macrophages.

Some observations suggest that gut flora plays a central role in driving inflammatory responses in inflammatory bowel disorders like pouchitis, Crohn’s disease and inflammatory bowel syndrome (6). A possible alternative strategy to fight these diseases may involve the manipulation of the local microbiome by probiotics in order to restore the microbial balance (7).

A controlled, double-blind, randomised study (8) on the efficacy of Lactobacillus plantarum 299V in patients with irritable bowel syndrome showed that with regards to all irritable bowel syndrome symptoms an improvement was noted in 95% of patients in the probiotic group versus 15% of patients in the placebo group. This study enrolled forty patients which received the probiotic at 20x10⁹ cfu/day) or the placebo for 4 weeks.

Other researchers conducted a study in a specific strain (SAM P1/Yit) of mice that develop inflammatory bowel disease (9). They showed that administration of fermented milk to mice reduced histological injury score compared with those in saline-treatment or unfermented milk-treated mice. The milk fermented with Bifidobacterium breve, Bifidobacterium bifidum and Lactobacillus acidophilus seems to be beneficial for the treatment of murine inflammatory bowel disease.
Prevention of hyperoxaluria by probiotics

Hyperoxaluria is a major risk factor for renal stones and in most cases, it appears to be sustained by increased dietary load or increased intestinal absorption. Previous studies have shown that endogenous digestive microflora, in particular Oxalobacter formigenes, utilize oxalate in the gut, thus limiting absorption (10). A multidisciplinary team from Italy tested the hypothesis of whether oxaluria can be reduced by means of reducing intestinal absorption through feeding a mixture of probiotics (11). Six patients with idiopathic calcium-oxalate urolithiasis and mild hyperoxaluria received daily for 4 weeks a mixture containing $8 \times 10^{11}$ of a mixture of freeze-dried L. acidophilus, L. plantarum, L. brevis, S. thermophilus and B. infantis during. The urinary excretion of oxalate was determined at the end of the study period and one month after ending the treatment. The treatment resulted in a great reduction of the urinary excretion of oxalate in all the six patients enrolled: means were 33.5±15.9 mg/24h at the end of the month of treatment and 28.3±14.6 mg/24h one month after the end of treatment, compared with baseline values 55.5±19.6 mg/24h (p<0.05). Results obtained in vitro showed that L. acidophilus and S. thermophilus degrade oxalate effectively but their growth was somewhat inhibited by oxalate. B. infantis showed a quite good degrading activity and their multiplication is not affected by oxalate. Of course, these results have to be strengthened by statistical significance resulting from trials enrolling higher number of individuals. Nevertheless, the authors postulate that the biological manipulation of the endogenous digestive microflora can be a novel approach for the prevention of urinary stone formation.

A possible antioxidative role of probiotics: an in vitro result

The oxidation and oxidative process of Low Density Lipoprotein (LDL) are believed to be important in arteriosclerosis. The antioxidative activities of various foods (vegetables, tea, wine...) have been investigated to find whether these activities inhibited oxidation of LDL. The in vitro results published by the Meiji Milk products showed that an extract of Streptococcus thermophilus 1131 and Lactobacillus delbrueckii subsp. bulgaricus 2038 inhibited the oxidation of rabbit erythrocyte membranes and human LDL (12). Neither the factor bearing this antioxidative property in probiotics nor the effect in vivo have been identified yet.

Antitumour effects of a probiotic on a murine model

A team from The Yakult Central Institute for Microbiological Research investigated the antitumour potential of an instillation of heat killed Lactobacillus casei Shirota on the murine bladder tumour (13). The probiotic was intravesically instilled once daily for 10 days starting on the day after implantation of the tumour bladder (MBT-2). Tumour appearance, bladder weight as well as local cellular immunity in bladder mucosa were observed on day 21 after tumour implantation. In the tested mice, the rate of tumour appearance is reduced compared with controls. The probiotic instillation is also accompanied by an increase of the local expression of antitumour cytokine messenger RNA (interferon $\gamma$ and Tumour Necrosis Factor $\alpha$). Therefore, it seems that L. casei Shirota instillation lead to inhibition of the growth of tumour cells in mice bladder. This effect is, at least partially, mediated by cytokines. According to the authors, intravesical instillation of this probiotic may represent potent immunotherapy for prophylaxis against bladder tumour recurrence.
A bacteriocin like factor released by *Lactobacillus bulgaricus*

Apart from lactic acid, lactic bacteria may produce a number of other substances with the potential to suppress the growth of spoilage bacteria or other strains of the same species. Particularly, an ever-increasing number of bacteriocins are being isolated. In addition to these substances, lactic acid bacteria may produce metabolites such as H$_2$O$_2$, organic acids, or ethanol that may cause growth inhibition.

A team from the Institut National de la Recherche Agronomique, France revealed the presence of at least three inhibitory substances in the culture supernatant fluid of *Lactobacillus delbrueckii* spp *bulgaricus* VI1007: H$_2$O$_2$, a bacteriocin-like molecule and a factor able to inhibit the growth of *Streptococcus thermophilus* (14).

These results show that the inhibiting effects of *Lactobacillus delbrueckii* spp *bulgaricus* VI1007 are important enough to be of ecological importance in the competition between strains and especially to prevent the growth of a second strain.

...and an antibiotic by *Bacillus subtilis*

*Helicobacter pylori* infection is the major cause of chronic gastritis and peptic ulcer disease in humans. The limited number of antibiotics and antibiotic resistance jeopardize the success of treatment. The use of probiotics as therapeutic agents against *H. pylori* is a studied opportunity.

In an *in vitro* essay (15), *Bacillus subtilis* 3 was found to inhibit *H. pylori*. The antagonistic activity of the probiotic is related to two compounds detected by thin-layer chromatography and high-performance liquid chromatography. One of these compounds was identified as anticoumacin A, an antibiotic with anti-inflammatory properties.

Health benefits of Milk and dairy products

Hans Meisel from the Federal Dairy Research Centre, Germany presented an overview on the specific biochemical properties and possible dietary pharmaceutical applications of peptides derived from bovine milk proteins. This review (15) that consider 117 references highlights the primary classes of bioactive milk peptides, their tropho-functional properties and their potentials application in milk-based foods.
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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Intestinal microflora plays a crucial role in the genotoxicity of the cooked mutagen 2-amino-3-methylimidazo(4,5-f) quinoline.
Carcinogenesis 22(10), 1721-1725.

Recombinant Lactobacillus plantarum inhibits house dust mite-specific T-cell responses.

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The 13C/2H-glucose test for determination of small intestinal lactase activity.