The organisms most commonly used as probiotics in dairy foods belong to the genera Lactobacillus and Bifidobacterium, which are food-grade organisms witnessing a long history of safe use. Some clinical reports that have associated certain Lactobacillus strains with rare cases of human infection have been reviewed in several papers in the last years; all of them concluded that probiotic lactobacilli and bifidobacteria are unlikely to pose any risk to consumers.

A new item in safety assessment of probiotic bacteria is now under evaluation: the presence of acquired and transferable antibiotic resistance genes (ABR). First of all it seems worthwhile to point out that at the moment drug resistance in lactobacilli and bifidobacteria is a safety issue according to the precautionary principle approach only. There are no data on the real contribution of food-borne, antibiotic resistant lactococci and streptococci to the widespread presence of drug resistance genes in pathogens, therefore a real risk assessment is not possible.

On the other hand we cannot ignore that since the ’80s the presence of plasmid encoding for drug resistance has been published and since that time numbers of papers have reported the isolation and characterization of genes encoding for antibiotic resistance in lactobacilli and, to a lesser extent, in bifidobacteria. The possible risk that probiotic bacteria can transfer antimicrobial resistance traits to gut microbiota cannot be entirely excluded. These observations prompted a range of reactions. The Scientific Committee on Animal Nutrition (SCAN), in charge of evaluating probiotics for animal feeding according to EU regulations has asked since 1999 to applicants to provide evidences that strains submitted for approval were not harbouring “acquired resistances”. Unfortunately no reference methods for phenotypic assessment or microbiological breakpoints value were available at that time. The EU interest in the assessment of the presence of drug resistances in non lactic acid bacteria is not only still alive but also growing. The EFSA panel named FEEDAP (which has replaced SCAN) is delivering every year new documents on this problem. More, the EFSA approach to the establishment of the Qualified Presumption of Safety (QPS) of micro-organisms in Food and Feed has specifically stated that also for micro-organisms granted of the QPS status, a case-by-case assessment will be required making specific reference to: “acquired antibiotic resistance determinants in lactic acid bacterium”.

The Expert Consultation on probiotics jointly convened by FAO and WHO has pointed out that: “it is recommended that probiotic bacteria should not harbour transmissible drug resistance genes encoding resistance to clinically used drugs”. This recommendation has suggested to FAO to continue to work in the area of probiotics with a newly created FAO/IDF/ISO Joint Action Team on probiotics. This action team consists of four project groups working on the assessment of probiotic microorganisms; one of them is dealing with methods to determine antibiotic resistance (http://www.fao.org/ag/agn/food/food_probiocons_en.stm) and the other three have been aimed to develop phenotypic and genetic tools to identify the presence of ABR in lactobacilli, bifidobacteria, lactococci and Streptococcus thermophilus. In this project a culture collection of little more than 1300 strains belonging to 20 species. All of them were identified by means of molecular taxonomy tools and also typed at the strain level, to avoid handling twice or more the same strain. Standardised phenotypic methods have been developed, providing relevant information on the presence of “atypical resistance” in a little more than 1% of the used strains. All these atypical resistances are now under evaluation for the presence of specific ABR genes as well as multi Drug non specific transporters.

Conjugal transferability has been showed for some of these ABR. Data produced up to now do confirm the presence of ABR in lactic acid bacteria and provide tools for further investigation, planned to provide ecological data on the percentage of presence of ABR lacstaic acid bacteria in food. Moreover, phenotypic and genetic tools develop in the frame of project could be used develop a safety assessment during the selection process of new probiotic strains.

A probiotic reduces allergies in children

Although it is well accepted today that certain probiotics have a positive effect on childhood allergies, the mechanism(s) involved remain unknown.

Recently, a clinical study of 53 children suffering from moderate to severe atopic dermatitis showed that taking Lactobacillus fermentum PCC (2×10^9 CFU/day for 8 weeks) resulted in reduced severity of the allergy (1).

A further study was conducted by the same research group in order to find a basis for these observations in the immune system (2). Mononuclear lymphocytes taken from subjects during the first clinical study were stimulated with different antigens:
- Vaccine antigens (diphtheria or tetanus toxoids)
- Allergens such as egg ovalbumin or milk β-lactoglobulin
- Staphylococcus aureus antigens (enterotoxin B [SEB] or heat-killed bacteria)
- Heat-killed L. fermentum
- A polysaccharide mitogen (phytohemagglutinin [PHA])

Production of pro- (IFNγ and TNFα) or anti-inflammatory cytokines (IL-5, IL-6, IL-10 and IL-13) by these lymphocytes was analyzed in blood samples taken 8 weeks before and after administration of the probiotic, then 8 weeks after consumption was stopped.

The results show that stimulation by non-specific antigens (PHA or SEB) caused a significant increase in the production of IFNγ by lymphocytes taken from children who had taken the probiotic. The increase was detected both at the end of the consumption period and 8 weeks later. The production of IFNγ, however, did not change in response to the specific stimuli tested (food allergens or anatoxins). Increased production of TNFα was also observed in response to each of the heat-killed bacteria (S. aureus or L. fermentum) at the end of the period of probiotic administration. After this date, a return to the base concentration of TNFα was reported. No other antigen modified production of this factor.

Among the anti-inflammatory cytokines tested, only production of IL-13 was affected by one of the stimuli. The concentration of this interleukin did in fact fall significantly in response to ovalbumin for the lymphocyte samples taken at the end of the probiotic administration period. However the effect did not last. This result is therefore interesting given the pro-allergic effect of IL-13.

In this study, an increase in IFNγ production in response to SEB enterotoxin was observed in the lymphocytes of subjects in whom the severity of the dermatitis had regressed the most. In this way, the authors highlighted a proportional relationship between the clinical effect of the probiotic and the ability of the lymphocytes to produce IFNγ in response to a non-specific stimulation.

Furthermore, the beneficial effect of the probiotic on the symptoms of dermatitis was associated with activation of Th1 type lymphocytes, revealed by an increase in production of the IFNγ pro-inflammatory cytokine. Apart from a transitory response to ovalbumin, no lymphocyte response was observed for specific allergens. It therefore appears that the immune circuits via which the probiotic exerts its anti-allergic effect in the context of atopic dermatitis remain to be discovered.

Lactobacillus casei inhibits pathogen-induced pro-inflammatory signals in the enterocytes - hypothesis for an action mechanism

Probiotic strains are allegedly able to modulate intestinal inflammation caused by pathogens in the gut. An in vitro study has confirmed that Lactobacillus casei DN-114 001 has this property, by highlighting a mechanism to inhibit inflammation caused by Shigella flexneri (3).

Caco-2 type human gut cells were cultivated either with or without L. casei DN-114 001 then exposed to S. flexneri. The proportions of intra- and extra cellular pathogens were analyzed with or without pre-incubation with L. casei. The results demonstrated that the lactobacillus does not affect the pathogen’s infection of the cells. A study of the expression of the gut cell genes showed that L. casei DN-114 001 inhibits the transcription of several genes coding for pro-inflammatory factors activated by S. flexneri. More precisely, L. casei inhibits the NF-κB pathway, probably by inhibiting degradation of the IkBα protein by the ubiquitin-proteasome complex. This mechanism has furthermore been described for commensal bacteria (4).

It should be remembered that the NF-κB transcription factor is activated by numerous stimuli such as stress, cytokines and mitogenic agents. It regulates the transcription of numerous genes involved in the inflammation process, especially those of inflammatory cytokines.

This study demonstrates the anti-inflammatory ability of the L. casei DN-114 001 probiotic in vitro and proposes an action mechanism. If this effect were confirmed by clinical studies, steps could be taken to use this probiotic to treat inflammatory gut disorders in humans.

One of the main health claims of probiotics is that they improve several gastrointestinal parameters. A recent study asked the question whether these parameters are modulated in healthy adults (5).

The study was conducted randomized and double-blind. Thirty healthy adults, suffering from no particular digestive problems before the study, were given daily either a standard yoghurt used as the control (Streptococcus thermophilus 10^5 CFU and Lactobacillus bulgaricus 4x10^6 CFU) or a fermented milk containing S. thermophilus, Lactobacillus coryniformis CECT5711 and Lactobacillus gasseri CECT5714 (10^8 CFU, 2x10^9 CFU and 2x10^9 CFU respectively). The tested product was administered for 4 weeks; for the two weeks before and after the study, the subjects followed a diet where all dairy products were excluded.

Changes in intestinal comfort as felt by the subjects were graded according to the following principle: On a scale from 0 to 10 - before the study the score was 5. The subjects then gave a score that compared the intestinal function after the study to this initial score. The subjects all reported any symptoms of gastric discomfort. Several objective parameters were also analyzed:

- Haematocrit and the composition of the blood lipids at the start and end of the period when the probiotic supplement was administered.
- Number of lactic bacteria and detection of probiotic strains in the faeces (weekly analysis).
- Biochemical properties of the faeces: Concentration of short chain fatty acids, pH, water content, enzyme activity, cytotoxicity (weekly analysis).

Consuming the products did not cause any side effects. There were no changes in the results of the blood tests after ingestion of the products. At the end of the administration period, the number of bacteria in the faeces was significantly higher in the tested group compared to the group receiving the yoghurt; two weeks later, the number had fallen and reached a similar value to that observed in the control group. The two lactobacilli tested were found in the faeces at the end of the fermented milk administration period. More short chain fatty acids were produced in the tested group than in the control group. The other physiological parameters analysed did not reveal any significant differences between the two groups. The subjects in the group given the fermented milk noted an improvement in their intestinal functions whereas no difference appeared for the yoghurt group.

The fermented milk resulted in temporary colonization by Lactobacillus coryniformis CECT5711 and Lactobacillus gasseri CECT5714 bacteria, associated with an increase in the number of lactic bacteria in the faeces. This study therefore supports the hypothesis according to which probiotics, or at least some probiotics, could improve the intestinal comfort of healthy subjects. It also shows that all probiotics do not provide this benefit; it may depend on the type of probiotic, the strain or probiotic mixture used.

Fate of Lactobacillus casei DN-114 001 in the digestive tract

The effects, or some of the effects, of probiotics on the host depend on the survival of the bacteria in the digestive system as well as their interaction with gut microflora. The Lactobacillus casei DN-114 001 probiotic was the subject of a twofold study (6).

The authors analyzed the composition and bacterial microflora of healthy adults consuming daily 3 x 10^10 CFU of L. casei DN-114 001 contained in fermented milk (fermented with Streptococcus thermophilus, Lactobacillus bulgaricus and L. casei DN-114 001). Samples of the faeces were obtained from each subject before the product was taken (D0), after it had been taken for 10 days (D10), and 10 days after consumption was stopped (D20). The subjects did not follow a special diet during the 4 weeks before the study, except for restrictions in the amount of fermented dairy products consumed - fewer than 2 products per week.

Quantification by "real time PCR" showed that by day 10, the population of bacteria in the group L. paracasei (including L. casei DN-114 001) had increased on average 1260 times in the faeces as compared to day 0. Although an analysis of the samples obtained on day 20 showed that the population decreased after consumption stopped, 75% of subjects still had a population level that was greater than their respective levels measured on D0. Although analysis of the samples using the PCR-RTGE and/or FISH techniques showed that the diversity of the L. paracasei was modified by ingesting the fermented milk, no variation in the structure of the dominant anaerobic microflora was however observed (in particular for the Clostridium cocoides, Faecilbacterium prausnitzii, Bacteroides and Bifidobacterium groups). Furthermore, the activity of the intestinal microflora, assessed by measuring the activity of several bacterial enzymes in the faeces, was not modified by the fermented milk.

This study of healthy subjects shows that consumption of fermented milk containing S. thermophilus, L. bulgaricus and L. casei DN-114 001 temporarily affects only the population of the L. paracasei group, and has no effect on the other bacteria groups. Detection of a large quantity of the DNA of L. paracasei bacteria in the faeces led the authors to believe that L. casei DN-114 001 apparently survives well during transit, at least in the upper parts of the digestive tract. However, although the molecular techniques used made it possible to measure the persistence of the bacterial DNA in the digestive tract, they do not make it possible to reasonably estimate the survival of the bacteria.

Globally, the fermented milk used in this study did not affect the homeostasis of the gut microflora. As several studies have already suggested for other probiotics, L. casei DN114 001 only remains in the host temporarily, during the consumption phase. To take advantage of the potential health effects of the probiotic, it therefore appears important to consume it continuously.
**Lactobacillus rhamnosus GG and growth of neonates**

A recent study indicates that *Lactobacillus rhamnosus* GG could offer interesting nutritional properties for neonates (7).

A randomized, placebo-controlled, prospective study was conducted double-blind on 105 healthy newborn babies aged between 0 and 2 months on their inclusion. Until they were 6 months old, they were fed either formula supplemented with LGG or standard formula. For breast-fed infants, the administered milk product had to correspond to at least half of their food intake. Upon enrolment and then on a monthly basis, a clinical examination collected statistics relating to the infants’ growth and information provided by the parents regarding digestion, crying and any illnesses. The faeces of 25 infants chosen randomly were analyzed at the start and end of the study.

Between the start and end of the study, growth (height), weight gain and head circumference were seen to be significantly greater in the group given the probiotic compared to the control group. The probiotic therefore appeared to favour growth. However, despite the fact the subjects were put into the two groups randomly, the height, weight and head circumference of the babies in the control group were significantly greater on enrolment. In their analysis, the authors put forward two arguments that indicate that this bias does, finally, not affect the validity of the result. Firstly, after making adjustments for the height and weight at birth, the differences observed between the two groups at the end of the study remain significant. Furthermore, although it is acknowledged that infants grow more quickly during their first year of life when their body weight at birth is lower than average, the weight of each of the infants taking part in the study was in all cases greater than average. This compensatory phenomenon therefore does not come into play for the sample studied.

Analysis of the results also shows that the occurrence of infections was similar in both groups. The probiotic appears to have been well tolerated and faeces were more frequent than in the control group. In the infants who received the LGG, the percentage of subjects colonized by lactobacilli was significantly higher than that observed in the control group. The authors do not specify whether the increased population of lactobacilli reflects the fact that LGG was also present or results from an increase in the presence of indigenous lactobacilli.

Milk enriched with LGG appears to favour growth but the bias in the methodology of this study tempers the conclusions relative to growth. This result needs to be confirmed by other clinical studies. The mechanism behind this potential beneficial effect remains to be clarified.

**Other effects of probiotics**

Certain beneficial effects of probiotics are allegedely based on their interactions with gut pathogens. A study has proposed an action mechanism for *Lactobacillus reuteri* RC-14 when fighting *Staphylococcus aureus* (8) infections.

In a previous study conducted on rats, it was seen that when *L. reuteri* RC-14 (formerly known as *L. fermentum*) and *S. aureus* were injected simultaneously at the site of surgery, infection by *S. aureus* was inhibited (9). To clarify the mechanism behind the interaction of the two bacteria, the authors of a new study performed co-culture experiments. The device used allowed the strains to develop in two different compartments separated by a membrane through which soluble compounds could diffuse. The membrane proteomes of *S. aureus* were analyzed in three culture conditions: co-culture with *L. reuteri* RC-14, co-culture with a lactobacillus that remained ineffective against *S. aureus* infection in the model studied in rats and finally *S. aureus* cultivated alone. The only significant change observed in the protein profile of *S. aureus* concerned a large fall in production in the presence of the SSL11 protein in the presence of *L. reuteri* RC-14. This protein is part of a group of staphylococcus specific antigens that would appear to be involved in making these bacteria so virulent.

Complementary experiments have shown that the supernatant fluids of *L. reuteri* RC-14 prevent activation of the SSL11 promoter, and also stop this promoter from working if it is already active. Characterization of this *L. reuteri* RC-14 signalling molecule is currently underway; the authors are attempting to isolate it.

The RC-14 strain of *L. reuteri* secretes a molecule that inhibits activity of the promoter of an antigen responsible for the virulence of *S. aureus*. In rats, this effect of *L. reuteri* RC-14 inhibits *S. aureus* infections caused by surgery. These results prepare the way in humans for a new method of fighting against opportunist *Staphylococcus aureus* infections occurring after surgery.

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**Lactobacillus casei** activates the innate immune system in the gut of mice

Different mechanisms could be involved in the activation of the immune system by probiotics. After administration of *Lactobacillus casei* CRL 431 to mice for 2, 5 or 7 days, the most obvious modifications occurring were an increase in activation of the innate immune cells accompanied by an increase in two specific markers of these cells: CD-206 receptors (mannose receptor) and TLR-2 (receptor involved in recognition of Gram+ bacteria and mycobacteria). However, no changes in the number of T lymphocytes could be measured.

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Anorexia nervosa - yoghurt improves certain immune system parameters

A randomized, double blind study was conducted to evaluate the impact of eating yoghurt on anorexic teenagers in the renutrition phase and on healthy teenagers (total of 65 subjects). Compared to the control product (skimmed milk) and after ten weeks of intake, the yoghurt caused an increase in the production of CD8+ lymphocytes and of IFNγ by lymphocytes in the two groups of subjects. This effect could be particularly beneficial to subjects suffering from anorexia nervosa, in whom depression of the immune functions is seen.

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Failure of VSL#3 to treat colitis in mice

It has already been shown that the VSL#3 probiotic mixture has properties to fight gut inflammation in humans and animals. In a study on mice whose aim was to find an action mechanism, this effect could not be reproduced. The probiotic administered to animals suffering from chemically-induced (by DSS) colitis changed the composition of the gut microflora but neither countered inflammation nor modified the types of mucus or the thickness of the mucus barrier. As other studies had already shown the efficacy of VSL#3 against inflammatory bowel diseases, the authors conclude that the efficacy of VSL#3 must be dependent on the agent inducing the colitis and the administration protocol.

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Consumption of *Lactobacillus GG* by mothers modulates the bacterial microflora of neonates

A study focusing on 53 mother-infant pairs has shown that consumption by mothers of *Lactobacillus GG* before and after birth influences the establishment of the gut microflora in their infants. The differences observed are related to the abundance and composition of the bifidobacteria pool. Those infants whose mothers were given the probiotic, had a more diversified bifidobacteria microflora, more rich in *Bifidobacterium breve* and less rich in *Bifidobacterium infantis* than in those infants whose mothers were given a placebo. Furthermore, compared to the control group, the microflora of these infants showed more differences with the mothers’ microflora.

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A probiotic fighting the intestinal effects of stress

A study on rats has shown that consuming *Lactobacillus farciniminis* reduces colic hypersensitivity and increases colic permeability resulting from stress. Two elements explaining the action mechanism have been put forward. Firstly, nitrogen monoxide produced spontaneously by the bacterium is probably involved. Secondly, the bacterium inhibits phosphorylation of a protein involved in the contractions of the cytoskeleton (p-MLC) and causes increased intestinal permeability.

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Bibliographic selection


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e.mail : syndifrais@syndifrais-syndilait.org