AFSSA provides a methodological framework for probiotic-related health claims

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Since so many products now on the market - both foods or food supplements - include bacteria (in particular lactic bacteria) and polysaccharides (in particular fructo-oligosaccharides and inulin), the AFSSA (French Agency for Food Safety) has drawn up a list of current knowledge about probiotics. This will make it easier to judge the claims made of their effects on humans.

The analysis has resulted in the publication of a report that can be read on the Agency's Web site: (http://www.afssa.fr/Ftp/Afssa/28500-28501.pdf).

The report begins with a short summary of the ecology of the digestive tube. This is an area where our knowledge is changing rapidly, including concepts that one might have once thought fundamental. The bacterial population of the gut (microbiota) has indeed proven itself to be not yet well known. The recent application of molecular biology tools has revealed that more than 80% of the faecal biomass cannot be cultivated. The intestinal microbiota appears to be composed of about one hundred different dominant species, but the number of species common to several individuals appears to be very limited. This suggests that the microbiota forms a host-specific "bacterial consortium" that characterizes the microbiota very much like a fingerprint. The genetic diversity that this implies thus appears considerable. This fact has both theoretical and practical consequences. From a theoretical point of view, it underlines the importance of the host's genotype on the composition of the microbiota and its stability over time and leads us to see the colonization of the gut by bacteria as the result of a selection or even a co-evolution (Backhed et al. 2005). It illustrates how much the concept of normal flora, in practical terms, is unrealistic.

The report then draws up a list of the effects attributed to probiotics. It is not surprising that it includes the phenomenon of functional complementation that they exert on the digestion of lactose - well established in lactose-deficient patients or short intestine syndrome patients - that distinguishes their consumption from the ingestion of other lactose-enriched products (heat-treated milk or fermented milks). Other more general effects are also cited, for example those concerning intestinal transit. It is more innovative to recognize that all the studies carried out on humans converge on the idea that certain strains induce innate immunity modulation and that adaptive immunity could also be strengthened by it, notably by a potentiation of the response of secretory IgA against pathogenic, bacterial or viral agents.

This analysis also points out, sometimes by default, a certain number of quality criteria that both the claims themselves and any validations of their justification should take into account. In reality this is the most interesting point of this work, in so far as that the state of the knowledge it addresses cannot be held to be final. On the contrary, everything now points to the fact that it will be subject to major upheavals during the next few years.

Several simple ideas predominate. The majority of effects relating to the ingestion of probiotics appear to be dependent on the bacterial strain in question. The benefits (if any) of a particular strain cannot be extrapolated to another. This implies that, in humans, validation studies must be conducted with the strain corresponding to the claim being made. In the same way, effects shown with products that contain live micro-organisms cannot be extrapolated to others containing heat-killed micro-organisms. In fact, if some studies have shown that some physiological effects could be due to metabolites or DNA from bacterial preparations, others have only obtained results when live microbial preparations were present.

In short, claims must be supported by studies conducted on humans under the same conditions the claims were originally made under and complying with the methodological rules generally implemented in clinical research.

* We are not concerned here with chapters focusing on prebiotics.
** A species is said to be dominant if it represents at least 1% of the total bacteria population.

Peptides formed by a probiotic increase osteogenesis in rats

Certain beneficial effects of probiotics may be explained by the production of peptides produced during the fermentation of milk proteins. Studies have indicated that the peptides isoleucyl-prolyl-proline, IPP and valyl-prolyl-proline, VPP, may increase the bioavailability of calcium (1). Two recent studies have highlighted the effect of IPP and VPP peptides on bone growth.

In the first study (2), researchers analyzed in vitro the influence of different products on the growth of osteoblasts* and the differentiation of osteoblast precursor cells*. The results highlighted an increase in osteoblastic bone formation in the presence of milk fermented by Lactobacillus helveticus LBK-16H or the active peptides it produces (IPP and VPP). No significant effect was obtained, on the other hand, on osteoclasts.

The same team then verified whether this effect could be reproduced in vivo (3). For 14 weeks, young rats suffering from bone growth disorders were given one of the following products: the whey of a milk fermented with L. helveticus LBK-16H, milk fermented with L. helveticus and Saccharomyces cerevisiae (produces the same peptides as L. helveticus LBK-16H, but in smaller quantities), milk fermented with Lactococcus sp. (does not produce the peptides under study), skinned milk or water. The parameters observed were bone density and bone mineral content in relation to the body mass of the animals.

Bone mineral density and content were greatest in the group receiving the milk fermented with L. helveticus LBK-16H. The difference was significant compared to water and skinned milk (p<0.01 for each product) and to milk fermented without bioactive peptides (p<0.05).

These results show that milk fermented with L. helveticus LBK-16H improves bone growth in rats and that IPP and VPP peptides are probably involved in this beneficial effect. Given the high stakes for public health of calcification problems in Western populations, this study promises a way of fighting osteoporosis, if its results are confirmed in humans. Therefore, the consumption of some fermented milks would appear to be doubly beneficial in terms of calcium intake and its assimilation by the body.

Consumption of fermented milks may reduce the risk of cardiovascular diseases

The links between food and health are today widely substantiated. For example, the role played by diet is taken into account in the aetiology of cardiovascular diseases (4). This has been confirmed by an American study (5) that analyzed the relationship between a cardiovascular risk marker - levels of homocysteine in the blood - and the components of the diet including milk and fermented milk products.

Let us remember that homocysteine metabolism is influenced by, among others, group B vitamins. Studies have shown that high values of homocysteine in the bloodstream are associated with an increased risk of cardiovascular disease.

The data this study is based on comes from a nationwide American survey - the "National Health and Examination Survey" (1988-1994). The sample is a sub-group of 5996 subjects, aged from 13 to 89. Certain populations, such as pregnant women and diabetics, were excluded from the study. A statistical analysis of this data reveals that consumption of milk or fermented milk* is inversely associated with serum concentrations of homocysteine (p=0.0029 and p=0.0218). Homocysteine levels are greater than 15.2 % in subjects who never drink milk as compared to those who drink it at least once a day and greater than 6.4 % in the absence of consumption of fermented milk compared to consumption of 15 or more products per month. Three other groups give similar results: cold breakfast cereals, peppers and cruciferous vegetables (cabbage, broccoli, turnips, etc.). The statistical independence of these different results has been checked.

These observations, made on almost 6000 subjects, show that the consumption of milk or fermented milk is significantly correlated to low serum levels of homocysteine, and therefore reduces cardiovascular risk. This effect may be due to the fact that these products are rich in riboflavin (vitamin B2), a vitamin involved in homocysteine metabolism. The authors conclude that the consumption of milk and fermented milks could play a role in reducing cardiovascular risk.

* The family studied is called "yoghurt" and includes frozen products. Note that in the US, the term "yoghurt" covers all fermented milks whether or not they contain live ferments.

This scientific letter "Yoghurts & fermented milks" is also available on the following website: www.maison-du-lait.com
Probiotics and allergy prevention - positive results in adults

Certain probiotics could provide health benefits other than to the digestive system, by protecting against allergic reactions (6). The results with the most support are currently those obtained with young children. They indicate that the administration of certain lactobacilli may reduce the incidence or severity of atopic eczema (7, 8). According to the most widely accepted hypothesis, this action could be linked to a change in the balance between two types of immune cells - T lymphocyte helper cells of type 1 and type 2 (Th1 and Th2) (9). A Japanese team has shown the benefits of probiotics on adult allergies (10).

This study, carried out double-blind and placebo-controlled, involved 49 adults suffering from chronic allergic rhinitis. For 8 weeks, these patients consumed daily either a milk fermented by Lactobacillus acidophilus L-92 (3x10^10 cfu/day) or a placebo. Two types of parameters were analyzed: immunity markers in the blood (levels of immunoglobulin E (IgE) and ratios of lymphocytes Th1/Th2) and the clinical benefits (if any). The ocular and nasal clinical symptoms were assessed, both by a doctor and the patients themselves who noted the symptoms that occurred and the medication used (self-medication if the symptoms became too unbearable).

Nasal symptoms and the associated use of medication were significantly reduced in patients taking the probiotic compared to the control group (p<0.05). The doctor’s observations confirmed this since both swelling and the colour of the nasal mucous were improved in the group given the fermented milk compared to the control group (p<0.05 for swelling and p<0.01 for colour). The patients also saw a reduction in ocular symptoms. However, the reduction was statistically insignificant (p<0.1). In the same way, for immune system markers, no statistically-significant difference was revealed between the two groups.

The results of this study show that consuming milk fermented with L. acidophilus L-92 can significantly alleviate the symptoms of patients suffering from chronic allergic rhinitis. However, despite a clear improvement in the clinical presentation, no modulation was observed in the immune system markers studied. Therefore, unlike what was seen in children, the beneficial effect of probiotics on allergy in adults does not include any modulation of the Th1/Th2 balance. Another mechanism, still to be elucidated, could be involved.

Preventing allergies via vaccination with a lactobacillus - proposal for a protocol

One way of using lactobacilli is to express an antigen on the bacteria in order to use them as an immunisation delivery vehicle. A recent publication presents an original variation on this principle. The idea is to propose protocol for vaccination against allergy using recombinant bacteria.

In patients suffering from allergies, one therapy consists in neutralizing immunoglobulin E (IgE), responsible for starting the atopic reaction, by injecting anti-IgE antibodies. To avoid the need for frequent injections with strong doses of anti-IgE, a Swiss team has suggested another strategy. This would consist in making the body produce its own anti-IgE antibodies, instead of supplying them to it. To do this, the body would be placed in contact with IgE-specific epitopes* in the form of antigens, which would naturally induce the production of anti-IgE antibodies (11). In this context, recombinant lactobacilli (that carry these epitopes) would be used as immunisation delivery vehicles.

In a preliminary study (12), this same team had isolated several epitopes characteristc of IgE. Tested on rhesus monkeys, these epitopes were seen to totally inhibit cutaneous allergic reactions.

The researchers created three recombinant strains using Lactobacillus johnsonii NCC2754: two expressed IgE epitopes and a third (used as a control) expressed a different protein to the IgE antigenic determinant. The production of anti-IgE antibodies was measured in mice that had been immunized using these recombinant bacteria via the subcutaneous or nasal routes.

The power of the immune response - evaluated from the levels of IgG specific antibodies in the IgE tested - varies depending on the epitope/administration route pairing. For each immunisation route, recombinant bacteria carrying IgG specific epitopes induce an increase in the production of anti-IgE antibodies that is statistically significant compared to the response of the control bacteria.

These results show that, in mice, it is possible to induce a significant anti-IgE response by the intra-nasal or subcutaneous administration of recombinant Lactobacillus johnsonii NCC2754 expressing an IgE epitope. It remains to be verified if the anti-IgE antibodies thus produced are effective in reducing allergic symptoms in atopic patients. These results open up a use for lactobacilli.

* Epitope or antigenic determinant: structure of the surface of the antigen, with which the antibody combines.

References:
Probiotics effective against ulcerative colitis

Ulcerative colitis is a chronic inflammation of the colon. An inadequate immune response vis-à-vis the gut microflora may play a role in triggering and maintaining this inflammation (13). In patients suffering from ulcerative colitis, clinical studies have indicated that probiotics can prevent relapses (14, 15, 16). Two recent studies indicate that certain probiotics may also be beneficial during the active phases of the disease.

The first study focuses on the effect of a symbiotic given double-blind for one month (and placebo controlled) to 16 adult patients in the inflammatory phase of ulcerative colitis (17). The product tested is an association of the probiotic Bifidobacterium longum (4x10^11 cfu/j) and the probiotic Synergy1 (inulin et oligofructose). Pre-existing medical treatment with steroids, immunosuppressive agents or NSAIDs (5-aminosalicylic acid) was continued during the study. The clinical parameters were evaluated with two scores reflecting the clinical symptoms (diarrhoea and discomfort) and the biopsies of the colon respectively. The level of expression of several immune system markers was also taken into account: the defensins specifically overexpressed in the active phase of ulcerative colitis and pro-inflammatory cytokines (TNFα and IL-1α).

At the end of the study, no significant difference was revealed for the clinical parameters. On the contrary, in the patients given the symbiotic, the expression of the pro-inflammatory cytokines and the defensins was significantly reduced at the end of the study (p<0.04). The TNFα and IL-1α rates for the treatment group were significantly lower than in the control group (p<0.02).

It therefore appears that administering the symbiotic for one month significantly reduces the expression of pro-inflammatory cytokines and defensins specific to the active phase of ulcerative colitis. The weakness of the effect on the clinical manifestations of the disease could be explained by an insufficient treatment period.

The second study (18) was a clinical trial conducted double-blind and placebo-controlled. Nineteen patients in the active phase of ulcerative colitis received either a milk fermented with different probiotics (Yakult strains): Bifidobacterium breve, Bifidobacterium bifidum and Lactobacillus acidophilus (of 1x10^11 bacteria per day), or a placebo. An anti-inflammatory treatment (5-aminosalicylic acid or sulfasalazine) was administered to all patients. The clinical state of the patients was determined, colon biopsies were performed, as well as an analysis of the short-chain fatty acid (SCFA) and bacteria content of the faeces.

At the end of the study, the clinical status of the patients receiving the fermented milk was significantly improved (p<0.05) compared to that of the control group. In the test group, the scores relating to the colon biopsies were also improved (p<0.01). In the same way, and only in the patients given the fermented milk, a significant increase in the faecal concentration of SCFA was observed (p<0.05).

These results indicate that the consumption of fermented milk improves the clinical status of patients during the active phase of ulcerative colitis, without causing any unpleasant side effects. This clinical benefit was accompanied by an increase in faecal SCFA that could be involved in the action mechanism of fermented milk.

These two pilot studies suggest a promising use of probiotics as an adjuvant to the traditional treatment of ulcerative colitis in the active phase.

Preventing infectious infantile diarrhoea with probiotics

The protection against diarrhoea that mothers give to babies is today well-documented (19). This effect may be linked to the specificity of the intestinal flora resulting from breast feeding (20). Administering probiotics to non breast-fed babies, with the goal of modulating the intestinal flora and therefore prevent infectious diarrhoea, has been successfully tested on numerous occasions (21, 22).

Since infants attending day-care centres are particularly exposed to the risk of respiratory and gastro-intestinal infections (23, 24), an Israeli team chose to study the impact of administering probiotics to this type of population (25).

The experimental design included 194 infants (4-10 months). Over 12 weeks, they received a standard baby formula (control group) or one that was supplemented with either Bifidobacterium lactis Bb-12, or Lactobacillus reuteri 55730. The height, weight and head circumference were measured regularly. Fever and respiratory and gastro-intestinal symptoms were recorded throughout the study. Parents reported any absences from the day-care centre, visits to the doctor or doctors’ prescriptions.

The administration of probiotics caused no unpleasant side effects or differences in growth in comparison with the control milk. In the infants receiving milk supplemented with probiotics, fever and diarrhoea were less frequent than in the control infants (p<0.001). Diarrhoea also did not last as long in the two groups taking probiotics (p<0.001). L. reuteri 55730 caused additional beneficial effects compared to the control group and the group receiving B. lactis Bb-12: reduction in the number of doctor’s appointments, prescriptions of antibiotics, absences from day-care or days with fever (p<0.037 in all cases). For the parameters describing respiratory infections, no differences were observed between the groups.

Administration of B. lactis Bb-12 or L. reuteri 55730 to infants in day-care centres reduced the occurrence and duration of infectious diarrhoea. These results, obtained from a large number of patients, confirm the conclusions of previous studies confirming the advantages of probiotics in managing the risk of infectious diarrhoea in infants.

Preventing infectious infantile diarrhoea with probiotics

Two probiotics protect against necrotizing enterocolitis in newborns

Necrotizing enterocolitis is a serious inflammation of the gastro-intestinal tract than essentially affects premature babies. A Chinese study of 367 premature babies, (birth weight <1500 g) showed that a probiotic mixture (the product Infioran®, composed of Lactobacillus acidophilus and Bifidobacterium infantis) reduces the incidence and severity of necrotizing enterocolitis. Doctors in Wisconsin have commented that this promising clinical study should be reiterated in order to confirm these results. New studies could also help to find the protecting mechanism involved.

A symbiotic preventing carcinogenesis in rats - towards an action mechanism

Compared to the administration of either a probiotic mixture (Lactobacillus rhamnosus GG and Bifidobacterium lactis Bb12) or a prebiotic (Synergy 1), the administration of a symbiotic (probiotic + prebiotic) reduces the frequency of chemically-induced tumours in rats. At the same time as preventing carcinogenesis, administering a symbiotic modulates the immune functions of Peyer's patch cells (lymph nodules of the intestinal mucous membrane). The authors put forward the hypothesis that the intestinal lymph tissue of the Peyer's patch cells may be involved in the inhibiting effect on carcinogenesis caused by the symbiotic.

Safety of probiotics - bifidobacteria remain sensitive to antibiotics

Sensitivity to antibiotics is a prerequisite that probiotics must comply with in order to remain innocuous. A French team has tested the resistance to antibiotics of fifty strains of bifidobacteria, isolated in humans, animals and food. All these strains showed weak resistance to a set of 30 antibiotics. These results confirm there is no risk in using bifidobacteria in human foods - the bacteria did not contain antibiotic-resistant genes and the risk of transferring resistance to pathogenic bacteria seems negligible.

Lactobacillus infections - what are the implications for probiotics?

A retrospective review of 200 cases of human infections has been conducted based on data published in the scientific literature between 1950 and 2003. About 30% of cases of mortal infections was associated with the presence of lactobacilli. In these clinical cases, mortality was not directly attributable to lactobacilli, the patients in question were immunodepressed and therefore sensitive to opportunistic infections. The authors of this review emphasize that in cases of endocarditis (where lactobacilli were present), the rate of mortality was statistically higher when it was a polymicrobial infection or inadequately treated bacteremia. Only a very small percentage (1.7%) of the listed cases of infection was associated with high levels of consumption of dairy products.

What regulations should govern probiotics?

One review has reported on European regulations concerning the use of probiotics in human and animal foods. Probiotics used in animal feeds are subject to regulations that require in vitro and in vivo safety tests that attest to the bacteria's sensitivity to antibiotics and to the absence of harmful metabolite production. Probiotics for human consumption, on the other hand, are not subject to any regulations in Europe, unless they are considered as new foods. To limit the number of studies to be conducted, the author believes that future regulations must lie somewhere a need for safety and a recognition of the harmlessness of bacteria that have been used in human foodstuffs for decades. In this context, a work group formed by the members of different European Commission committees (Scientific Committee on Animal Nutrition, Scientific Committee on Food and Scientific Committee on Plants) has suggesting drawing up a list of probiotics that are presumed safe in accordance with the concept of “Qualified Presumption of Safety”. This would consist of drawing up a set of criteria with which the micro-organism would need to comply.

References:
Bibliographic selection


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