Bundesinstitut
für gesundheitlichen Verbraucherschutz und Veterinärmedizin

Final report of the BgVV Working Group "Probiotic Microorganism Cultures in Food"

October 1999

1. Introduction

At the initiative of the Group of Experts for Food Hygiene and Veterinary Medicine (*Arbeitskreis Lebensmittelhygienischer Tierärztlicher Sachverständiger* [ALTS]), a working group entitled *Probiotic Microorganism Cultures in Food* was set up at the Federal Institute of Health Protection of Consumers and Veterinary Medicine (*Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin* [BgVV]) in September 1997.

The group was formed against the backdrop of the emergence of "probiotic" dairy products which were for the first time noticed on the market in the mid-1990s. These products are claimed to impart certain health benefits to the human body.

With regard to the legality of the claims, which the control authorities have seen as being in contravention of the regulations on misleading claims or health-related claims* (Article 17(1)5 and Article 18 of the German Food and Commodities Act (*Lebensmittel- und Bedarfsgegenständegesetz* [LMBG]), the way in which these products have been advertised has led to discrepancies between the views held by the food control authorities and those of the producers.

Other questions - a few are listed below as examples - haven arisen in terms of assessing these products from a scientific point of view:

- What is meant by probiotic microorganism strains and products made from them?
- Are there differences between probiotic products and those made by conventional methods?
- What beneficial effects can be regarded as having a sufficiently credible scientific basis?
- What requirements for consumption of food are necessary in order that a particular kind of food product containing the bacteria concerned may be regarded as developing probiotic action?
- Are the strains of microorganisms used in probiotic foods safe for human consumption?

The working group consisting of scientists, business people and representatives of food control authorities and consumer associations had been set up with the objective of answering all those open questions on aspects other than legal ones.

The results of discussions conducted mainly by sub-groups of the working group are set forth below.

2. Definitions of terms

Probiotics are used in food products, drugs and animal feed.

The following definitions have been established for their use as dietary supplements in food products:

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* More appropriately: disease-related claims as defined by Article 18 (1) 1, LMBG.

Probiotics:

Probiotics are certain living microorganisms; sufficient amounts of which reach the intestines in an active form to exert health benefits.

Prebiotics:

Prebiotics are specific indigestible substances which selectively support the growth of bifidobacteria and possibly other microorganisms in the intestines. They are beneficial to health.

Synbiotics:

Synbiotics are products in which probiotics and prebiotics are combined to produce a synergistically beneficial effect.

The probiotic properties of the cultures in question must develop in the food products advertised, i.e., it is not enough to demonstrate that the probiotic effects develop in the cultures. Hence, it was imperative to have a definition of the term "probiotic food", which was formulated as follows:

Probiotic food:

Probiotic food is food containing probiotics in an amount sufficient to produce probiotic effects when such food is ingested.

Products containing probiotic microorganisms are classified as either "foodstuffs" or "drugs" on the basis of existing legal provisions.

Food products containing probiotic cultures can be both foodstuffs for common consumption and dietary foods, provided they are in compliance with the provisions of Article 1 of the relevant EU framework directive (Council Directive 89/398/EEC of 3 May 1989 on the approximation of the laws of the Member States relating to foodstuffs intended for particular nutritional uses).

Probiotic cultures marketed for immediate consumption in isolated form are not regarded to be foodstuffs in Germany and consequently must not be classified as food supplements.

Since there are no uniform definitions of the terms "food" and "food supplements" in the EU, the existence of different rules in the member states may lead to distortion of competition between them.

3. Identity and safety of probiotic strains and their behavior in food

3.1 What microorganisms are added to food?

The oldest example of traditional "biotechnology" to preserve food is the lactic acid fermentation which has been known for thousands of years. Starter cultures which were used for making thick sour cream for the first time in Copenhagen and Kiel in 1890 marked the beginning of modern industrial microbiology and food technology.

Lactic acid bacteria are by far the most important form of microorganisms advertised as probiotic microorganisms. Strains belonging to the genera *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus* and *Streptococcus* are used in food products, and recently, strains of other genera have been added to the list.

Studies of probiotic products by the genera *Lactobacillus (L.)* and *Bifidobacterium (B.)* on the European market can be found in:

1. the "*L. acidophilus* group" and its species *L. acidophilus and L. johnsonii*;

2. the "*L. casei* group" according to its current taxonomy and the heterofermentative species *L. reuteri*;

3. *Bifidobacterium spp.: B. animalis, [B. bifidum]*[1]*, B. longum, B. lactis*[2]*, B. infantis* and *B. breve*. The form of this genus most frequently isolated from probiotic yogurt products in Germany was *B. animalis.*

3.2 Amount of probiotics used

The question of the minimum microorganism count in the product ("effective microorganism count") is of crucial importance with regard to the use of probiotics in food products and their intended health benefits.

There is still uncertainty as to the microorganism count required to achieve a beneficial effect. Human trials are normally based on a certain amount of intake of living microorganisms per day (and in some cases per kg of body weight). Owing to the lack of reliable clinical data, it is not deemed advisable to lay down the minimum microorganism count at this point and time. Other reasons why a count that would have general validity cannot be established are that factors such as persistence and site of action also have a bearing on the required microorganism count. Experience shows that physiologically effective metabolic bacterial action is relevant and measurable only if the number of microorganisms per gram of food is in excess of 10^6.

Depending on the amount ingested and taking into account the best-before date, a regular – in most cases daily – intake of 10^8 to 10^9 probiotic microorganisms is necessary to achieve probiotic action in the human organism.

3.3 Safety from the viewpoint of consumer protection

Lactic acid bacteria should not be used in food products, unless it has been ascertained that they are safe for human consumption. Meeting this general requirement becomes problematic when genera such as enterococci or individual *Lactobacillus* species include strains which manifest themselves as clinical isolates. Studies have shown, for instance, that certain strains of lactic acid bacteria occur in clinical isolates in people with impaired immune function. But in terms of their resistance behavior to antibiotics and other characteristics, these strains were not identical with strains contained in food.

In the case of many lactic acid bacteria, the decision to classify them as "safe" or "not safe" depends on the specific genetic makeup of the strain in question rather than that of the species in general and the consumer's state of health.

There has not been a single case of infection that could be traced back to the ingestion of food produced using lactic acid bacteria. On the other hand, certain routes of infection are conceivable which might circumvent the body's barriers (e.g. mucous membranes), enabling autochthonous microflora to be involved in the process. Microorganisms of the *L. casei* group and *Streptococcus spp.* have occasionally been found in the mouth flora of healthy individuals.

Epidemiological studies show that the incidence of individual strains of lactic acid bacteria in sick people is extremely low. Moreover, the use of these microorganisms as production cultures for food products over a period of more than 100 years is sufficient proof of the fact that they are safe.

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1 The label on food products using the term "*B. bifidum*" is an incorrect declaration; the microorganisms used here are usually *B. animalis* or *B. longum.*

2 In the case of *B. lactis*, this seems in all likelihood to be a synonym for *B. animalis* or *B. longum*.

The following physiological effects of probiotic microorganisms need to be examined for their safety:

- formation of biogenic amines (in particular, tyramine, histamine, phenylethylamine);
- activation of procarcinogens (with the aid of azo reductase, nitro reductase, ß-glucuronidase);
- induction and/or destruction of thrombi with the aid of specific hydrolases;
- activation of platelet aggregation;
- binding to fibrinogen and fibronectin;
- mucin reduction (detected in certain bifidobacteria; can be regarded as a condition for an invasion by microorganisms);
- hemolytic activity;
- transmissible resistances to antibiotics.

Further research is also needed for example on the capacity of deconjugation and dehydroxylation of bile salts.

3.4 Identification, detection and differentiation of probiotic microorganisms

Probiotic properties are strain-specific. The genus and species have to be known in order to identify the strain. In routine tests of probiotic food products, it should suffice to identify the species. However, for this purpose manufacturers should be obliged to reveal their methods of identification and detection to the food control authority upon request. In isolated instances, a routine laboratory may be able to carry out a strain-specific identification, if this involves simple phenotypic (morphologic, physiological, biochemical) differentiation. Otherwise, strains can be differentiated only by specialized laboratories, which, on request, must be provided with the authentic strain to enable them to carry out the tests.*

3.5 Effects of food processing methods

For probiotics to be able to unfold their action in foodstuffs, they must survive in the food product concerned and preserve their probiotic qualities even after processing and storage up to the best-before date.

Among the factors that should be controlled in probiotic foodstuff production are the following ones: Culture cultivation, the food product matrix, the processing method, the food product's own microflora, the packaging used, the best-before date, etc.

The product should have a demonstrable functional effect on the human body as confirmed by scientific studies. In such studies, the description of the product should embrace all relevant parameters, which are necessary for their beneficial health effects to develop throughout a product’s shelf life up to the best-before date. These should include the correct scientific name and the population density of the probiotic strain, specific activities relevant to the effects they are claimed to have. These specifications are used to define the products and product categories which are claimed to have certain effects. The proof must be based on scientific evidence obtained from human studies.

Proof of the advertised action must be furnished using the product, i.e., it is not enough merely to demonstrate the effect of its probiotic ingredient or probiotic substance. If the efficacy of a substance has been demonstrated in earlier scientific studies, it should also be shown that this efficacy is under no restriction in the product in question. An additional evaluation of efficacy is not necessary when minor changes are made to the product, such as adding aromatic substances and flavoring agents, which do not impair the beneficial

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* This can be done, for instance, by depositing the strains in a culture bank accessible to the laboratories.

effects the product is claimed to have. But a renewed examination is required if changes are made (e.g., substantial changes in the formulation of the substrate fermented by the probiotic strain) for which there is no proof that the product's efficacy is retained. The effect must be reproducible.

Proof of efficacy should be furnished with reference to normal eating habits, i.e., the quantity normally consumed by the target group in a population should be taken into consideration.

4. Effects of probiotic microorganisms in the human body

As probiotic microorganisms are credited with special health benefits, such claims should be examined for their credibility and scientific evidence. Results obtained *in vitro* or in animal studies can be used for screening purposes or they can provide evidence as to the existence of possible health effects. However, only effects which have been documented in studies on the human body can be regarded as providing sufficient scientific proof. It is also important to show that the same effects are brought about in the product concerned.

Findings made on individual strains cannot be arbitrarily transferred to apply to other strains. This holds true in particular for mixed cultures whose properties must be tested in such combinations. They cannot simply be inferred from the individual strains used.

In human trials of probiotic microorganism cultures, defined, clearly characterized strains of bacteria should be used. The trials should be randomized, double blind and backed up by placebo controls. The placebos used in such trials should be in the form of similar products without probiotic properties. The results should be reproducible and checked using scientific methods, e.g., by publishing the results in recognized scientific periodicals.

In the following, we shall deal with the current state of scientific knowledge on the probiotic action of microorganisms in the human body based on some data on effects relevant to health.

- **Passage of probiotic microorganisms through the gastrointestinal tract**

The passage of live probiotic bacteria through the upper gastrointestinal tract is seen as the primary prerequisite for achieving probiotic action in the human body. Some direct human studies have been conducted using special small intestine probes to study microorganism passage into the terminal ileum and caecum. "Worst-case model" studies are frequently conducted using a pH of 2 and bile acid. Gastric-juice and bile-salt tolerance and sufficient resistance to digestive enzymes are decisive for the microorganisms' survival during the passage through the stomach and upper intestinal tract, and, in turn, for their adherence to cells of the small intestine and formation of colonies in the colon. The ability to survive is strain-dependent, but it also depends on the composition of the food as whole. For instance, the ability to survive the passage through the gastrointestinal tract is better in milk.

The effects of probiotic microorganisms on the composition and function of the intestinal flora depends not only on the flora's stability, but also on the type of diet. Food rich in dietary fiber, for instance, promotes fermentation and hence formation of short-chain fatty acids. There is a manifest increase in bifidobacteria and apparent cryptae in the intestinal mucosa as a result of increased butyrate formation.

It is not clear what bearing intermittent ingestion of probiotic food has on intestinal flora. The effect wears off very rapidly (a few days) after regular intake of such food is stopped, and after three weeks it is no longer detectable.

- **Influence of probiotic microorganisms on milk sugar utilization in lactase deficient humans**

The fact that persons with lactose intolerance tolerate both conventional and probiotic yogurts containing comparable amounts of lactose better than milk is regarded as being founded on sufficiently reliable scientific evidence. The beneficial effect of a product can be cancelled through heat sterilization. The decisive criterion is the presence of ß-galactosidase activity in the microorganisms involved in degrading milk sugar, thereby at least in part compensating for the lactase deficiency. This also explains why not all yogurts are equally well tolerated, for instance, when the probiotic strain from the L. casei group is lactase-negative. It is not likely that lactase-deficient humans tolerate probiotic milk products better than yogurt products.

- **Influence of probiotic microorganisms on gastrointestinal infections – prevention of infections through oral intake of probiotic microorganisms**

The influence of probiotic microorganisms on certain forms of diarrhea in children caused by rotavirus and by clostridia after broad-spectrum antibiotics treatment is seen as being confirmed. Studies on the prevention of traveler's diarrhea have so far proved negative. Displacement and suppression of pathogenic microorganisms through agglutination or competition for certain receptors on the cell surface, which prevent enterovirulent strains from penetrating the mucosa cell, are being discussed as the underlying effective mechanisms. To obtain information on the prevention of gastrointestinal infections through the oral intake of probiotic microorganisms, *in vivo* trials on humans need to be conducted by the manufacturers of the product. Merely showing that there is a shift in the microbial spectrum is not in itself sufficient evidence for claiming preventive action.

- **Modulation of the immune response**

Modulation of the immune response is not necessarily synonymous with immune system enhancement. But human trials suggest that certain lactic acid bacteria strains exert a positive effect on the immune system, e.g., through an induced increase in certain immunoglobulins such as sIgA, increased non-specific phagocytose activity or increased formation of certain non-proinflammatory cytokines.

- **Influence of probiotic microorganisms on the activity of bacterial enzymes in the lower gastrointestinal tract, possible influences on carcinogens in the colon**

As far as the effect of probiotic microorganism cultures on the activity of cancer-promoting enzymes in the lower gastrointestinal tract and on lowering the activity of some harmful metabolic products is concerned, animal experiments and initial human trials suggest a probable protective effect against colon carcinoma. But no long-term studies have been conducted to indicate the minimum effective concentration necessary to reduce the risk of colon cancer. Considerable research into this question is still necessary.

5. Claimed effects of probiotic food

Article 17 (Prohibitions for Protection against Deception) and Article 18 (Prohibition of Health Claims[*] of the German Food and Articles of Daily Use Act (LMBG)) are the primary legal basis for labeling probiotic food products. The wording of these Articles corresponds to that of the Council Directive 79/112/EEC on the Approximation of the Laws of the Member States relating to the Labeling, Presentation and Advertising of Foodstuffs for Sale to the Ultimate Consumer.

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[*] More appropriately: disease-related claims as defined by Article 18 (1) 1, LMBG.

Under Article 17(1)5a, LMBG, a claim is misleading when food products are said to have effects for which there is no scientific evidence or insufficient scientific evidence. Under Article 18(1)1 of LMBG, it is prohibited to make claims relating to the alleviation of symptoms or prevention of diseases.

For this reason statements falling under Article 18 of LMBG cannot be made unless the existing legislation is amended.

Food processors are keen on avoiding coming into conflict with Article 18 LMBG and therefore use expressions such as "strengthens the body's defenses" or "promotes health". This reflects the industry's efforts to circumvent the use of medical terms such as "immune system."

Probiotic qualities apply only to food products containing minimum concentrations of certain cultures. Claimed positive effects of probiotic food on the human body, which may well be true in specific instances, must be stated in concrete terms in order not to violate the provisions on misleading claims under Article 17, paragraph 1, 5a of the LMBG.

As far as claims are concerned, it impossible to make general statements as to what is permissible and what is not. Each case should be decided on its own merits. It is up to the manufacturer who claims that his products have probiotic qualities to provide sufficiently reliable scientific evidence to substantiate such claims. If the reliability or scientific evidence of such claims is challenged, the courts must decide.

6. Conclusion

- A BgVV working group has defined the terms probiotics and probiotic food and drawn up a report on their identity, safety, special properties and effects. Criteria for determining the effects of probiotic microorganisms on the human body have been established. The working group expressed its support for openness in the interests of properly understood consumer protection.
- The report can be used by consumers, food control authorities, manufacturers and commercial establishments to assess probiotic food on the basis of uniform criteria.
- Regular intake of live microorganisms contained in probiotic food is necessary to achieve certain effects.
- There is sufficient evidence to support the fact that the lactic acid bacteria traditionally used in food are safe for human consumption. Criteria for assessing the safety of probiotic microorganisms and establishing their effects on health are set forth in the report.
- In terms of consumer protection, the benefits and risks of product cultures should be carefully assessed. Similarly, sufficient scientific evidence must be provided to substantiate claimed health effects.
- As a prerequisite for both product development in the European Economic Area and providing legal security for the consumer, it is important that a uniform legal basis be developed to cover questions of declaration, claims and other aspects associated with probiotic food.
- It is proposed to present this report to the European Commission.