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# Probiotics in food

## Health and nutritional properties and guidelines for evaluation

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# Foreword

The beneficial effects of probiotic foods on human health and nutrition are increasingly recognized by health professionals. Recent scientific work on the properties and functionality of living micro-organisms in food have suggested that probiotics play an important role in immunological, digestive and respiratory functions, and that they could have a significant effect on the alleviation of infectious diseases in children and other high-risk groups. In parallel, the number and type of probiotic foods and drinks that are available to consumers, and marketed as having health benefits, has increased considerably.

In view of this growing popularity of probiotic foods, and the lack of international consensus on the methodology to assess their efficacy and the safety, FAO and WHO initiated work to examine the scientific evidence on the functional and safety aspects of probiotics in food. In particular, an expert consultation on the health and nutritional properties of powder milk with live lactic acid bacteria was convened by FAO and WHO in Cordoba, Argentina in 2001, and an expert working group organized in 2002 to develop guidelines for the evaluation of probiotics in food.

The FAO/WHO consultation in 2001 brought together international scientific experts to evaluate available information on the functional and safety aspects of probiotics in powder milk. The consultation examined available scientific information on the dietary impact of probiotics, evaluated their properties, benefits, safety and nutritional features, and considered their potential adverse effects, taking into consideration work done by national authorities, FAO, WHO and other international organizations and relevant global fora. It reviewed the scientific basis for health claims linked to probiotic foods, considered regulatory needs and discussed strategies for the safety and nutritional assessment of probiotics, taking into account public concerns and food safety evaluation findings. The consultation generated a number of recommendations for further research, as well as priorities for the evaluation of safety and nutritional aspects of probiotics and regulatory requirements.

In follow-up to this consultation, FAO and WHO convened an expert working group to develop Guidelines for the Evaluation of Probiotics in Food. The resulting Guidelines provide a methodology for use in the evaluation of probiotics, and define the criteria and specific levels of scientific evidence needed to make health claims for probiotic foods.

By supporting the development of scientific knowledge on the functional and safety aspects of probiotics, FAO and WHO hope to enhance the overall safety and quality of food for consumers. In particular, it is hoped that the outputs of the FAO/WHO expert consultation and working group on probiotics will be used as a science-based assessment process for managerial decisions on probiotics, and that the Guidelines for the Evaluation of Probiotics in Food will provide a practical model to scientifically evaluate probiotics and be adopted by industry. It is also expected that these outputs will be useful for national work on health and nutrition claims, and as a scientific assessment of a novel food.

# *Health and Nutrition Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria*

**Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria**

**Cordoba, Argentina**
**1-4 October 2001**

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## Annex 1: List of Participants

# 1. Introduction

A joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) Expert Consultation on Health and Nutritional Properties of Powder Milk with Live Lactic Acid Bacteria was held in the Amerian Cordoba Park Hotel, Cordoba, Argentina from 1 to 4 October 2001. The Consultation, which was the first meeting of this group, focused on the evaluation of the scientific evidence available on the properties, functionality, benefits, safety, and nutritional features of probiotic foods. A total of 11 experts from 10 countries participated in the Consultation. The complete list of participants is given in Annex 1.

Mr Juan Schiaretti, Minister of Production of the Province of Cordoba, opened the Consultation. He acknowledged the need for sound scientific evidence to substantiate health benefits associated with probiotic foods. Mr Victor Faraudo, Secretary of Agriculture of the Province of Cordoba; Mr Carlos Debandi, President of the Cordoba Science Agency, and Mr Eduardo Echaniz, Coordinator of the National Codex Committee also gave welcome addresses. Dr Jorgen Schlundt and Dr Maya Pineiro spoke on behalf of the World Health Organization and the Food and Agriculture Organization of the United Nations. In their statements, the importance of probiotics to the health of the human population was indicated, with particular reference to their potential in developing countries.

The Consultation elected Dr Gregor Reid as Chairperson and Dr Catherine Stanton as Rapporteur.

# 2. Background

The beneficial effects of food with added live microbes (probiotics) on human health, and in particular of milk products on children and other high-risk populations, are being increasingly promoted by health professionals. It has been reported that these probiotics can play an important role in immunological, digestive and respiratory functions and could have a significant effect in alleviating infectious disease in children.

As there are no international consensus on the methodology to assess the efficacy and the safety of these products, at present, it was considered necessary to convene an Expert Consultation to evaluate and suggest general guidelines for such assessments.

The Consultation evaluated the latest information and scientific evidence available on the functional and safety aspects of probiotics, as well as the methodology to assess such aspects, by bringing together worldwide scientific experts in the field.

# 3. Scope

The Consultation agreed that the scope of the meeting would include probiotics and prebiotics in food, and exclude reference to the term biotherapeutic agents, and beneficial microorganisms not used in food. The Consultation has redefined probiotics for the purpose of this meeting as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host', but restricted its scope to discussion of 'Live microorganisms which when consumed in adequate amounts as part of food[1] confer a health benefit on the host'. The Consultation agreed that the specific issues related to powder milk could not be discussed without a more general consideration of probiotics in food.

The Consultation agreed to confine its discussion to the following:

a) Properties of probiotic strains and their assessment
b) Probiotic product specifications, quality assurance and regulatory issues
c) Safety and beneficial human health effects

As background to these discussions, the Consultation received background papers and presentations on:

- Taxonomy and physiology of lactic acid bacteria, effects and function on nutrition (Morelli L);
- Technological and commercial applications of lactic acid bacteria; Health and Nutritional Benefits in Dairy Products (Gilliland S);
- Regulatory and clinical aspects of dairy probiotics (Reid G).

The Consultation focused on strains available as probiotics in food. Although the Consultation did not specifically address issues related to genetically modified organisms, the concepts and principles are equally applicable to all probiotics. The potential importance of probiotic strains used in animal feeds as they pertain to human health was recognized.

# 4. History of Probiotics

The term probiotic is a relatively new word meaning "for life" and it is currently used to name bacteria associated with beneficial effects for humans and animals. The original observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff, the Russian born Nobel Prize winner working at the Pasteur Institute at the beginning of the last century, who suggested that "The dependence of the intestinal

[1] Water is included as a food

microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (Metchnikoff, 1907).

At this time Henry Tissier, a French paediatrician, observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y-shaped morphology. These "bifid" bacteria were, on the contrary, abundant in healthy children (Tissier, 1906). He suggested that these bacteria could be administered to patients with diarrhea to help restore a healthy gut flora.

The works of Metchnikoff and Tissier were the first to make scientific suggestions concerning the probiotic use of bacteria, even if the word "probiotic" was not coined until 1960, to name substances produced by microorganisms which promoted the growth of other microorganisms (Lilly and Stillwell, 1965). Fuller (1989), in order to point out the microbial nature of probiotics, redefined the word as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance". A similar definition was proposed by Havenaar and Huis in 't Veld (1992), "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora". A more recent, but probably not the last definition is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma, 1998).

It is clear that these definitions have:

1) restricted the use of the word probiotic to products which contain live microorganisms;
2) pointed out the need for providing an adequate dose of probiotic bacteria in order to exert the desirable effects.

The observations of Metchnikoff and Tissier were so appealing that commercial exploitation immediately followed their scientific works. Unfortunately, results were not always positive and most of these observations were anecdotal. The probiotic concept was therefore regarded as scientifically unproven and it received minor interest for decades, with some research involving animal feeding, in order to find healthy substitutes for growth promoting agents. In the last 20 years however, research in the probiotic area has progressed considerably and significant advances have been made in the selection and characterization of specific probiotic cultures and substantiation of health claims relating to their consumption.

Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used, but not exclusively, as probiotic microorganisms and a growing number of probiotic foods are available to the consumer. Some ecological considerations on the gut flora are necessary to understand the relevance, for human health, of the probiotic food concept.

Bacteria are normal inhabitants of humans (as well as the bodies of upper animals and insects) including the gastrointestinal tract, where more than 400 bacterial species are

found (reviewed by Tannock, 1999): half of the wet weight of colonic material is due to bacterial cells whose numbers exceed by 10-fold the number of tissue cells forming the human body. Normally the stomach contains few bacteria ($10^3$ colony forming units per ml of gastric juice) whereas the bacterial concentration increases throughout the gut resulting in a final concentration in the colon of $10^{12}$ bacteria/g. Bacterial colonization of the gut begins at birth, as new-borns are maintained in a sterile status until the delivery begins, and continues throughout life, with notable age-specific changes (Mitsuoka, 1992). Bacteria, forming the so-called resident intestinal microflora, do not normally have any acute adverse effects and some of them have been shown to be necessary for maintaining the well being of their host.

As an example of the beneficial role of intestinal microflora, it is possible to cite what has been referred to as "colonization resistance" or "barrier effect" (van der Waaij et al., 1971; Vollaard and Clasener, 1994) meaning the mechanism used by bacteria already present in the gut to maintain their presence in this environment and to avoid colonization of the same intestinal sites by freshly ingested microorganisms, including pathogens. Therefore, it could be assumed that dietary manipulation of gut microflora, in order to increase the relative numbers of "beneficial bacteria" could contribute to the well being of the host. This was also the original assumption of Metchnikoff who however, cautioned that:

"Systematic investigations should be made on the relation of gut microbes to precocious old age, and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body."

This prudent statement can still be regarded today as an invitation to scientists to investigate the probiotic bacteria in more depth and with care.

# 5. Guidelines for the Assessment of Probiotic Microorganisms

In order to assess the properties of probiotics, the Consultation suggested that the following guidelines be used. For use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut. This means they must be resistant to gastric juices and be able to grow in the presence of bile under conditions in the intestines, or be consumed in a food vehicle that allows them to survive passage through the stomach and exposure to bile. They are Gram positive bacteria and are included primarily in two genera, *Lactobacillus* and *Bifidobacterium* (Holzapel et al., 1998; Klein et al., 1998).

## *5.1 Selection of probiotic strains for human use*

Probiotics must be able to exert their benefits on the host through growth and/or activity in the human body (Collins et al., 1998; Morelli, 2000). However, it is the specificity of the action, not the source of the microorganism that is important. Indeed, it

is very difficult to confirm the source of a microorganism. Infants are born with none of these bacteria in the intestine, and the origin of the intestinal microflora has not been fully elucidated. It is the ability to remain viable at the target site and to be effective that should be verified for each potentially probiotic strain.

There is a need for refinement of *in vitro* tests to predict the ability of probiotics to function in humans. The currently available tests are not adequate to predict the functionality of probiotic microorganisms in the intestine.

## *5.2 Classification and identification of individual strains*

Classification is the arranging of organisms into taxonomic groups (taxa) on the basis of similarities or relationships. Nomenclature is the assignment of names to the taxonomic groups according to rules. Identification is the process of determining that a new isolate belongs to one of the established, named taxa.

The Consultation recommended that probiotics be named according to the International Code of Nomenclature to ensure understanding on an international basis. The Consultation strongly urged that for the sake of full disclosure, probiotic strains be deposited in an internationally recognized culture collection.

Since probiotic properties are strain related, it is suggested that strain identification (genetic typing) be performed, with methodology such as pulse field gel electrophoresis (PFGE). It is recommended that phenotypic tests be done first, followed by genetic identification, using such methods as DNA/DNA hybridization, 16S RNA sequencing or other internationally recognized methods. For the latter, the RDP (ribosomal data base project) should be used to confirm identity (www.cme.msu.edu/RDP/).

## *5.3 Defining and measuring the health benefits of probiotics*

A number of health effects are associated with usage of probiotics. There are differing degrees of evidence supporting the verification of such effects and the Consultation recognizes that there are reports showing no clinical effects of certain probiotic strains in specific situations (Andersson et al. 2001). While a rigorous review of each topic was not within the scope of the Consultation, an attempt was made to provide guidelines on parameters for measuring health benefits.

The use of probiotic microorganisms to confer health benefits on the host must indicate the dosage regimens and duration of use as recommended by the manufacturer of each individual strain or product based upon scientific evidence, and as approved in the country of sale. While this practice is not currently in place, the Consultation strongly recommended that each product should indicate the minimum daily amount required for it to confer specific health benefit(s). Such evidence should, where possible result from *in vitro*, animal (where appropriate) and human studies. Examples have been cited below to illustrate studies on specific strains and clinical outcomes. In doing so, the emphasis

should not be on one particular strain being termed as superior to another, rather that the benefit conferred and the methods used to obtain and measure said benefits are of most importance.

### 5.3.1 Disorders associated with the gastrointestinal tract

#### 5.3.1.1 Prevention of diarrhea caused by certain pathogenic bacteria and viruses

Infectious diarrhea is a major world health problem, responsible for several million deaths each year. While the majority of deaths occur amongst children in developing countries, it is estimated that up to 30% of the population even in developed countries are affected by foodborne diarrhea each year. Probiotics can potentially provide an important means to reduce these problems. It should be noted that some of the studies referenced below utilize probiotics administered in a non-food form.

The strongest evidence of a beneficial effect of defined strains of probiotics has been established using *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB-12 for prevention (Saavedra et al., 1994; Szajewska et al., 2001) and treatment (Isolauri et al., 1991; Guarino et al., 1997; Majamaa et al., 1995; Shornikova et al., 1997; Perdone et al., 1999; Guandalini et al., 2000) of acute diarrhea mainly caused by rotaviruses in children.

In addition to rotavirus infections, many bacterial species cause death and morbidity in humans. There is good *in vitro* evidence that certain probiotic strains can inhibit the growth and adhesion of a range of enteropathogens (Coconnier et al., 1993, 1997; Hudault et al., 1997; Gopal et al., 2001; Bernet Camard et al., 1997), and animal studies have indicated beneficial effects against pathogens such as *Salmonella* (Ogawa et al., 2001; Shu et al., 2000). There is evidence from studies on travelers' diarrhea, where some of the causative pathogens have been presumed to be bacterial in nature, that benefits can accrue with probiotic administration (Hilton et al., 1997).

It is important to note that probiotic therapy of acute diarrhea should be combined with rehydration if available. Current WHO recommendations state that clinical management of acute diarrhea should include replacement of fluid and electrolytes losses along with nutritional support (WHO, 1995). Oral rehydration salts (ORS) have been widely used in such disease management, and it is within this context that the combination therapy with probiotics is hereby advocated. Effects such as probiotic restoration of the non-pathogen dominated intestinal microflora secondary to infection, maintaining mucosal integrity and improving electrolyte balance could have a significant impact on programmes of treatment and prevention of acute diarrhea in developing countries.

A major problem associated with antibiotic treatment is the appearance of diarrhea, often caused by *Clostridium difficile*. This organism is not uncommon in a healthy intestinal tract, but the disruption of the indigenous microflora by antibiotics leads