Food and Agriculture Organization of the United Nations

World Health Organization

Health and Nutritional 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

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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 Amerian Córdoba Park Hotel, Córdoba, 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.

The Minister of Production of the Province of Córdoba, Mr. Juan Schiaretti opened the Consultation. He acknowledged the need for sound scientific evidence to substantiate health benefits associated with probiotic foods. The Secretary of Agriculture of the Province of Córdoba Mr. Victor Faraudo; the President of the Córdoba Science Agency, Mr. Carlos Debandi and the Coordinator of the National Codex Committee Mr. Eduardo Echaniz 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 recipient working at the Pasteur Institute at the beginning of the last century, who suggested that "The dependence of the intestinal 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).

[1] Water is included as a food

At this time Henry Tissier, a French paediatrician, observed that children with diarrhoea 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 diarrhoea to help restore a healthy gut flora.

The works of Metchnikoff and Tissier were the first to make scientific suggestions about 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 quite 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 characterisation 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 colonisation 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 colonisation 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 hybridisation, 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 practise 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 diarrhoea 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 food-borne 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 travellers' 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 programs 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 to an abnormal elevation of their numbers, and subsequent symptoms related to toxin production. The rationale therefore to use probiotics is that in such patients, administration of exogenous commensal microorganisms (that is probiotics) is required to restore the microflora to one

that more closely reflects the normal flora prior to antibiotic therapy. Some open ended studies have indeed shown that this approach can alleviate the signs and symptoms of *C. difficile* infection (Gorbach et al., 1987; Biller et al., 1995; Bennet et al., 1986). With respect to antibiotic-associated diarrhea, probiotics have proved useful as a prophylactic regimen, and potentially they can also be used to alleviate the signs and symptoms once antibiotic induced diarrhea has occurred (Arvola et al., 1999; Vanderhoof et al., 1999; Armuzzi et al., 2001). It must be recognized that evidence for therapeutic effects against *C. difficile*, and other disorders has been obtained using certain probiotic strains, such as *L. rhamnosus* GG. It is important to note that such effects may also be conferred by other strains, but scientific evidence may not yet be available or the microorganisms involved may not be included in the scope of this Consultation.

5.3.1.2. *Helicobacter pylori* infection and complications

A new development for probiotic applications is activity against *Helicobacter pylori*, a Gram negative pathogen responsible for type B gastritis, peptic ulcers and gastric cancer. *In vitro* and animal data indicate that lactic acid bacteria can inhibit the growth of the pathogen and decrease urease enzyme activity necessary for the pathogen to remain in the acidic environment of the stomach (Midolo et al., 1995; Kabir et al., 1997; Aiba et al., 1998; Coconnier et al., 1998). Human data is limited, but there is some evidence of an effect induced by *L. johnsonii* La1 (Michetti et al., 1999). In terms of measuring probiotic effects, feasible end points include the suppression of the infection (which may be reversible upon cessation of treatment), combination treatment with antibiotics leading to fewer side effects such as acid reflux, and lower risk of recurrent infection (Michetti et al., 1999; Canducci et al., 2000; Felley et al., 2001). Placebo-controlled trials are needed before specific claims can be made for probiotic anti-*Helicobacter pylori* benefits in humans with respect to prevention and treatment. Such studies are warranted given the preliminary evidence to support these effects.

5.3.1.3. Inflammatory diseases and bowel syndromes

Inflammatory bowel diseases, such as pouchitis and Crohn's disease, as well as irritable bowel syndrome may be caused or aggravated by alterations in the gut flora including infection (Shanahan, 2000). These are new avenues of investigation, although it is premature to state a firm action of probiotics in these conditions. Some studies support the potential role of probiotics in therapy and prophylaxis and illustrate that combinations of strains may have a role to play in remediation (Gionchetti et al., 2000; Gupta et al., 2000). The intestinal microflora likely plays a critical role in inflammatory conditions in the gut, and potentially probiotics could remediate such conditions through modulation of the microflora. Clinical and mechanistic studies are urgently required to better understand the interface between the microbes, host cells, mucus and immune defenses, and to create efficacious interventions. Such studies should include molecular examination of the intestinal (not only fecal) flora and long-term (5-10 years) effects of probiotic microorganisms.

5.3.1.4 . Cancer

There is some preliminary evidence that probiotic microorganisms can prevent or delay the onset of certain cancers. This stems from the knowledge that members of the gut microflora can produce carcinogens such as nitrosamines. Therefore, administration of lactobacilli and bifidobacteria could theoretically modify the flora leading to decreased β-glucuronidase and carcinogen levels (Hosada et al., 1996). Furthermore, there is some evidence that cancer recurrences at other sites, such as the urinary bladder can be reduced by intestinal instillation of probiotics including *L. casei* Shirota (Aso et al., 1995). *In vitro* studies with *L. rhamnosus* GG and bifidobacteria and an *in vivo* study using *L. rhamnosus* strains GG and LC-705 as well as *Propionibacterium* sp. showed a decrease in availability of carcinogenic aflatoxin in the lumen (El-Nezami et al., 2000; Oatley et al., 2000). However, it is too early to make definitive clinical conclusions regarding the efficacy of probiotics in cancer prevention.

The Consultation was not convinced that there is sufficient proof of a correlation between probiotics and specific anti-cancer effects, and urged that extensive studies are required. Such studies must utilize internationally recognized markers for cancer, or risk of cancer, and evaluate such markers and presence of carcinogenic lesions or tumors over a suitably long period of time for prevention of primary cancer, and reduction of the incidence of recurrences.

5.3.1.5. Constipation

The ability of probiotic therapy to alleviate constipation (difficulty in passing stool, excessive hardness of stool, slow transit through the bowel) is debatable, but may be a feature of selected strains. Randomized placebo controlled efficacy studies aimed at exploring these effects are strongly recommended.

5.3.2. Mucosal immunity

The innate and adaptive immune systems are the two compartments traditionally described as important for the immune response. Macrophages, neutrophils, natural killer (NK) cells and serum complement represent the main components of the innate system, in charge of the first line of defence against many microorganisms. However, there are many agents that this system is unable to recognize. The adaptive system (B and T cells) provide additional means of defence, while cells of the innate system modulate the beginning and subsequent direction of adaptive immune responses. Natural killer cells, including gamma/delta T cells, regulate the development of allergic airway disease, suggesting that the interleukins play an important role. Intravenous, intraperitoneal and intrapleural injection of *L. casei* Shirota into mice significantly increased NK activity of mesenteric node cells but not of Peyer's patch cells or of spleen cells (Matsuzaki and

Chin, 2000), supporting the concept that some probiotic strains can enhance the innate immune response.

A number of studies have been performed *in vitro* and in animals (Gill et al., 2000) which clearly show that probiotic strains can modify immune parameters. Correlating these findings with events taking place in the human body is still somewhat unclear, but evidence is mounting that such effects occur. In a series of randomized, double blind, placebo controlled clinical trials, it was demonstrated that dietary consumption of *B. lactis* HN019 and *L. rhamnosus* HN001 resulted in measurable enhancement of immune parameters in the elderly (Arunachalam et al., 2000; Gill et al., 2001; Sheih et al., 2001).

Probiotic modulation of host immunity is a very promising area for research. Supportive data is emerging, such as those carried out in humans showing that probiotic microorganisms can enhance NK cell activity in the elderly (Gill et al., 2001) and non-specific host defenses can be modulated (Donnet-Hughes et al., 1999; Perdigon et al., 1999).

There is a need to specify whether the activities being advocated are designed to operate in otherwise healthy people or subjects with known diseases. Some of the critical factors involved in the host's defenses have been identified and include the induction of mucus production or macrophage activation by lactobacilli signaling (Mack et al., 1999; Miettinen et al., 2000), stimulation of sIgA and neutrophils at the site of probiotic action (for example the gut), and lack of release of inflammatory cytokines or stimulation of elevated peripheral immunoglobulins (Kaila et al., 1992; Gardiner et al., 2001). It is also recognized that in some situations, stimulation of factors such as inflammatory cytokines may confer health benefits on the host.

Future studies should focus on the effect in humans, and elucidate the mechanisms of action within systems which simulate the *in vivo* situation, and link this to bacterial and human genomics.

5.3.3. Allergy

In a double-blind, randomized, placebo-controlled trial, *L. rhamnosus* GG was given to pregnant women for four weeks prior to delivery, then to newborns at high risk of allergy for six months with the result that there was a significant reduction in early atopic disease (Kalliomaki et al., 2001). This study illustrates the potential for probiotic microorganisms to modulate the immune response and prevent onset of allergic diseases. In other clinical studies with infants allergic to cow's milk, atopic dermatitis was alleviated by ingestion of probiotic strains *L. rhamnosus* GG and *B. lactis* BB-12 (Majamaa and Isolauri, 1996; 1997; Isolauri et al., 2000). The precise mechanisms have not been elucidated, but the premise is based upon the ability of lactobacilli to reverse increased intestinal permeability, enhance gut-specific IgA responses, promote gut barrier function through restoration of normal microbes, and enhance transforming growth factor beta and interleukin 10 production as well as cytokines that promote production of IgE

antibodies (Kalliomaki et al., 2001; Isolauri, 2001). Whether T-helper-1 (TH1) is enhanced and/or T-helper-2 (TH2) dominance is reduced remains to be determined, as do the time-points of these types of events. Certain microorganisms can contribute to the generation of counter-regulatory T-helper cell immune responses, indicating that use of specific probiotic microorganisms could redirect the polarized immunological memory to a healthy one (McCracken and Lorenz, 2001).

5.3.4. Cardiovascular disease

There is preliminary evidence that use of probiotic lactobacilli and metabolic by-products potentially confer benefits to the heart, including prevention and therapy of various ischemic heart syndromes (Oxman et al., 2001) and lowering serum cholesterol (De Roos and Katan, 2000). While the Consultation believes these findings to be important, more research and particularly human studies are required before it can be ascertained that probiotics confer health benefits to the cardiovascular system.

5.3.5. Urogenital tract disorders

Excluding sexually transmitted diseases, almost all infections of the vagina and bladder are caused by microorganisms that originate in the bowel. There is a strong correlation between presence of commensals, particularly lactobacilli in the vagina with health, and an absence of these microorganisms in patients with urogenital infections. Disruption of the normal vaginal flora is caused by broad-spectrum antibiotics, spermicides, hormones, dietary substances and factors not, as yet, fully understood. There is some evidence that probiotic microorganisms delivered as foods and topical preparations have a role in preventing urogenital tract disorders. The criteria for selection of effective probiotic strains have been proposed (Reid and Bruce, 2001) and should include verification of safety, colonization ability in the vagina and ability to reduce the pathogen count through competitive exclusion of adherence and inhibition of pathogen growth.

5.3.5.1. Bacterial vaginosis

Bacterial vaginosis (BV) is a disease of unknown etiology resulting from the overgrowth of various anaerobic bacterial species and associated with the disappearance of lactobacilli, which dominate the normal vagina. Many women with BV are asymptomatic yet are at risk of more serious complications such as endometriosis, pelvic inflammatory disease and complications of pregnancy including pre-term labour. There is some clinical evidence to suggest that oral and vaginal administration of lactobacilli can eradicate asymptomatic (Reid et al., 2001a; 2001b) and symptomatic BV (Hilton et al., 1995; Sieber and Dietz, 1998). Oral administration of *Lactobacillus acidophilus* and yogurt has been used in the prevention and therapy of candidal vaginitis, although no efficacy data have yet been generated (Hilton et al., 1992). The necessity for the lactobacilli to produce hydrogen peroxide has been proposed, but given that these microorganisms are more prone to being killed by spermicides, the combination of two or more strains, one of which produces hydrogen peroxide and others which resists spermicidal killing, may prove to be more therapeutic.

5.3.5.2. Yeast vaginitis

Yeast vaginitis is a very common ailment, often precipitated by antibiotic use, exposure to spermicides or hormonal changes as yet not fully understood. Unlike BV and urinary tract infection, yeast vaginitis is not necessarily due to loss of lactobacilli. Few *Lactobacillus* strains are able to inhibit the growth and adhesion of *Candida albicans* or other *Candida* species, and there is no solid evidence to indicate that intravaginal administration of lactobacilli can eradicate yeast infection. However, there is some evidence to suggest that lactobacilli ingestion and vaginal use can reduce the risk of recurrences (Hilton et al., 1992; 1995) and further studies are warranted since this disease is widespread and debilitating.

5.3.5.3. Urinary tract infections

Several hundred million women are affected by urinary tract infection (UTI) annually. Uropathogenic *Escherichia coli* originating in the bowel is the responsible agent in up to 85% of cases. Asymptomatic bacteruria is also a common finding in women, and sometimes it is followed by symptomatic UTI. There is evidence, including randomized controlled data to suggest that once weekly vaginal capsules of freeze dried *Lactobacillus* strains GR-1 and B-54 (Reid et al., 1995) prepared with addition of skim milk, and once daily oral capsule use of *Lactobacillus* strains GR-1 and RC-14 (Reid et al., 2001b), can result in the restoration of a lactobacilli dominated vaginal flora and lower risk of UTI recurrences. By creating a lactobacilli barrier in the vagina, it is believed that fewer pathogens can ascend into the bladder, thereby blocking the infectious process.

5.3.6. Use of probiotics in otherwise healthy people

Many probiotic products are used by consumers who regard themselves as being otherwise healthy. They do so on the assumption that probiotics can retain their health and well-being, and potentially reduce their long-term risk of diseases of the bowel, kidney, respiratory tract and heart. Several points need to be made on this assumption and its implications. The Consultation recognized that the use of probiotics should not replace a healthy lifestyle and balanced diet in otherwise healthy people.

Firstly, there is no precise measure of “health” and subjects may actually have underlying and undetectable diseases at any given time. Secondly, no studies have yet been undertaken which analyse whether or not probiotic intake on a regular basis helps retain life-long “health” over and above dietary, exercise and other lifestyle measures. One study of day care centres in Finland showed that probiotic use reduced the incidence of respiratory infections and days absent due to ill health (Hatakka et al., 2001). The Consultation would like studies to be done to give credibility to the perception that probiotics should be taken on a regular basis by healthy men, women and children. Such

studies should be multi-centred and require randomization on the basis of age, gender, race, nutritional intake, education, socio-economic status and other parameters.

It is currently unclear as to the impact of regular probiotic intake on the intestinal microflora. For example, does it lead to the depletion or loss of commensal microorganisms which otherwise have beneficial effects on the host? While there is no indication of such effects, the issue needs to be considered. Furthermore, the concept of restoring a normal balance assumes that we know what the normal situation in any given intestinal tract comprises. It was deemed important by the Consultation to further study the various contributions of gut microorganisms on health and disease. Another point worthy of note is that, to date, the ingestion of probiotic strains has not led to measurable long-term colonization and survival in the host. Invariably, the microorganisms are retained for days or weeks, but no longer (Tannock et al., 2000). Thus, use of probiotics likely confers more transient than long-term effects, and so continued intake appears to be required.

In newborn children, where a commensal flora has not yet been established, it is feasible that probiotic microorganisms could become primary colonizers that remain long-term, perhaps even for life. While such probiotic usage can prevent death and serious morbidity in premature, low birth weight infants (Hoyos, 1997), the alteration of flora in healthy babies is a more complex situation. Just so, an implication of the Human Genome Project is that selected probiotics may be used at birth to create a flora that improves life-long health. These issues are very important for the future, and will require full discussion including human ethical considerations.

6. Testing Methods for Establishing Health Benefits Conferred by Probiotic Microorganisms

Proper *in vitro* studies should establish the potential health benefits of probiotics prior to undertaking *in vivo* trials. Tests such as acid and bile tolerance, antimicrobial production and adherence ability to human intestinal cells should be performed depending on the proposed health benefit (Collins et al., 1998; Havenaar and Huis in't Veld, 1992).

In order to ascertain that a given probiotic can prevent or treat a specific pathogen infection, a clinical study must be designed to verify exposure to the said pathogen (preventive study), or that the infecting microorganism is that specific pathogen (treatment study). If the goal is to apply probiotics in general to prevent or treat a number of infectious gastroenteritis or urogenital conditions, the study design must define the clinical presentation, symptoms and signs of infection, and include appropriate controls.

For *in vivo* testing, randomized double blind, placebo controlled human trials should be undertaken to establish the efficacy of the probiotic product. The Consultation recognized that there is a need for human studies in which adequate numbers of subjects are enrolled to achieve statistical significance (Andersson et al., 2001). It would be

preferable to have such findings corroborated by more than one independent center. For some foods, it may be difficult to separate a probiotic effect from an effect related to the general product characteristics of the food. Therefore, it is essential that proper controls be included in these human trials. Furthermore, data obtained with one specific probiotic food cannot be extrapolated to other foods containing that particular probiotic strain or to other probiotic microorganisms.

With respect to measuring the health benefits in human studies, consideration should be given to clinically relevant outcomes in the population being studied. For diarrheal studies, this might be preventing death in some countries, while in others it might be prevention of a defined and statistically significant weight loss, decreased duration of watery\liquid stools, and faster recovery to normal health, as measured by restoration of normal bowel function and stool consistency.

Although it is known that certain probiotics can elicit beneficial effects (as discussed in Section 5), little is known about the molecular mechanisms of the benefits reported (Andersson et al., 2001). The mechanisms may vary from one probiotic to another (for the same benefit via different means) and the mechanism may be a combination of events, thus making this a very difficult and complex area. It could involve the production of a specific enzyme(s) or metabolite(s) that act directly on the microorganism(s), or the probiotic could also cause the body to produce the beneficial action.

Examples of possible probiotic mechanisms of action, in the control of intestinal pathogens include:

- Antimicrobial substance production
- Competitive exclusion of pathogen binding
- Competition for nutrients
- Modulation of the immune system

The Consultation proposes that clear experiments (*in vitro* and/or *in vivo*) should be designed at the molecular level to elucidate the mechanisms of probiotic beneficial effects. Appropriate experiments including genetic analysis to elucidate the mechanism of actions should be performed.

Probiotic bacteria containing β-galactosidase can be added to food to improve lactose maldigestion (Kim and Gilliland, 1983). However, a similar health effect is also observed for lactose fermenting starter bacteria such as *L. delbrueckii.* ssp. *bulgaricus* and *S. thermophilus* in fermented milk products like yogurt (Kim and Gilliland, 1984; Kolars et al., 1984). These traditional starters are not considered probiotics since they lack the ability to proliferate in the intestine (Klein et al., 1998).

7. Safety Considerations

7.1. Antimicrobial resistance profiles of probiotics

As with any bacteria, antibiotic resistance exists among some lactic acid bacteria, including probiotic microorganisms (Salminen et al., 1998). This resistance may be related to chromosomal, transposon or plasmid located genes. However, insufficient information is available on situations in which these genetic elements could be mobilised and it is not known if situations could arise where this would become a clinical problem.

There is concern over the use in foods of probiotic bacteria that contain specific drug resistance genes. Bacteria, which contain transmissible drug resistance genes, should not be used in foods. Currently, no standardized phenotypic methods are available which are internationally recognized for lactobacilli and bifidobacteria (non-pathogens). The Consultation recognizes the need for the development of standardized assays for the determination of drug insensitivity or resistance profiles in lactobacilli and bifidobacteria.

The Consultation is aware that plasmids exist in lactobacilli and bifidobacteria, especially in strains isolated from the intestine, which have genes encoding antibiotic resistance. Due to the relevance of this problem, it is suggested that further research be done relating to the antibiotic resistance of lactobacilli and bifidobacteria.

When dealing with selection of probiotic strains, it is recommended that probiotic bacteria should not harbour transmissible drug resistance genes encoding resistance to clinically used drugs. Research is required relating to the antibiotic resistance of lactobacilli and bifidobacteria and the potential for transmission of genetic elements to other intestinal and/or food borne microorganisms.

7.2. Safety of probiotics in humans

In terms of safety of probiotics, the Consultation believes that a set of general principles and practical criteria need to be generated to provide guidelines as to how any given potential probiotic microorganism can be tested and proven to have a low risk of inducing or being associated with the etiology of disease, versus conferring a significant health benefit when administered to humans. These guidelines should recognize that some species may require more vigorous assessment than others. In this respect, the evaluation of safety will require at least some studies to be performed in humans, and should address aspects of the proposed end use of the probiotic strain.

Information acquired to date shows that lactobacilli have a long history of use as probiotics without established risk to humans, and this remains the best proof of their safety (Naidu et al., 1999; Saxelin et al., 1996). Also, no pathogenic or virulence properties have been found for lactobacilli, bifidobacteria or lactococci (Aguirre and Collins, 1993). Having stated that, the Consultation acknowledges that under certain conditions, some lactobacilli strains have been associated with adverse effects, such as

rare cases of bacteremia (Saxelin et al., 1996). However, a recent epidemiological study of systematically collected lactobacilli bacteremia case reports in one country has shown that there is no increased incidence or frequency of bacteremia with increased usage of probiotic lactobacilli (Salminen et al., 2001).

It is also acknowledged that some members of lactic acid bacteria, such as enterococci may possess virulence characteristics. For this and other reasons, the Consultation recommends that *Enterococcus* not be referred to as a probiotic for human use. The rationale is based upon:

A. Strains can display a high level of resistance to vancomycin (Shlaes et al., 1989; Eaton and Gasson, 2001; Lund and Edlund, 2001), or can acquire such resistance. If this resistance is present, transfer to other microorganisms may occur and this could enhance the pathogenesis of such recipients (Noble et al., 1992; Leclercq and Courvalin, 1997).

B. Certain strains of vancomycin resistant enterococci are commonly associated with nosocomial infections in hospitals (Leclercq and Courvalin, 1997; Woodford et al., 1995).

The Consultation recognizes that some strains of *Enterococcus* display probiotic properties, and may not at the point of inclusion in a product display vancomycin resistance. However, the onus is on the producer to prove that any given strain cannot acquire or transfer vancomycin resistance or be virulent and induce infection.

8. Probiotic Product Specifications, Quality Assurance and Regulatory Issues

8.1. Regulatory issues

Government regulations differ among countries, however the status of probiotics as a component in food is currently not established on an international basis. For the most part, probiotics come under food and dietary supplements because most are delivered by mouth as foods. These are differentiated from drugs in a number of ways, especially with respect to claims. Drugs are allowed to claim effectiveness in the treatment, mitigation or cure of a disease, whereas foods, feed additives and dietary supplements can only make general health claims.

In order to understand where probiotic products currently fall in terms of regulatory agencies, and the claims that can be made with their use, the following US example is provided (www.fda.gov). Consumers are permitted access to products ingested as pills, capsules, tablets and liquids, or in capsules sold in health food stores or via the internet.

- A ‘health claim’ is defined as “a statement, which characterizes the relationship of any substance to a disease or health-related condition, and these

should be based upon well-established, generally accepted knowledge from evidence in the scientific literature and/or recommendations from national or international public health bodies. Examples include 'protects against cancer'.

- A structure/function claim is defined as "a statement of nutritional support that describes the role of a nutrient or dietary ingredient to affect the structure or functioning of the human body, or characterizes the documented mechanism by which a nutrient or dietary ingredient acts to maintain such structure or function. Examples include 'supports the immune system'. Claims that substances can treat, diagnose, cure or prevent a disease are not structure/function claims.

The Consultation recommends that disease reduction claims be permitted for specific probiotics if these have been demonstrated using guidelines outlined in this report.

The new paradigm of risk analysis is making its way into regulatory food safety and focuses on a functional separation of the science-based risk assessment and risk management. However, the issue of communication is now also considered an important integrated part of risk analysis. Communication includes exchange between assessors and managers and two-way interaction with other interested parties. Within this concept, the transparency of the decision making process for food safety regulatory action is emphasized, as well as the importance of providing a vehicle for consumers and others to participate in the development process. Therefore communication efforts relative to the use of probiotics should be considered as an integrated part of the development of regulatory initiatives.

8.2. Appropriate labeling

To clarify the identity of a probiotic present in the food, the Consultation recommends that the microbial species be stated on the label. If a selection process has been undertaken at the strain level, the identity of the strain should also be included, since the probiotic effect seems to be strain specific.

There is a need to accurately enumerate the probiotic bacteria in food products in order to include them on the label. The label should state the viable concentration of each probiotic present at the end of shelf-life (Reid et al., 2001c).

8.3. Manufacturing and handling procedures

To ensure that any given culture maintains the beneficial properties, the stock culture should be maintained under appropriate conditions and be checked periodically for strain identity and probiotic properties. Furthermore, viability and probiotic activity must be maintained throughout processing, handling and storage of the food product containing the probiotic, and verified at the end of shelf-life.

Adequate quality assurance programs should be in place. Good manufacturing practices should be followed in the manufacture of probiotic foods. The Consultation recommends that the Codex General Principles of Food Hygiene and Guidelines for Application of HACCP (CAC, 1997) be followed.

8.3.1. Powdered milk products

Since a purpose of this Consultation was to address the health and nutritional properties of milk powder with live lactic acid bacteria, it was considered necessary to further address the issue in this report. Methods of production of dried probiotic powders should be such that adequate numbers of viable probiotic bacteria are maintained in the dried powder following manufacture, and also retention/stability of probiotic properties should be ensured throughout shelf-life.

The Consultation agreed that there is not adequate information available on the stability of probiotics in powdered milk and little information is available on the issue of probiotic quality following spray drying. Cell damage and loss of viability of the probiotic culture occur during the spray drying process (Daemen and van der Stege, 1982; Gardiner et al., 2000). Thus improvements in spray drying methods are necessary to ensure better survival, including the use of protective agents which have been shown to enhance survival of lactobacilli (Prajapati et al., 1986; Selmer-Olsen et al., 1999) and environmental adaptation (Desmond et al., 2001). Probiotic stability during powder storage is inversely related to storage temperature (Gardiner et al., 2000), and methods have to be identified to address this. Although not published in the literature, certain companies producing starter cultures have the technology to produce freeze dried lactic acid bacteria including probiotics that are ‘stabilized’ and thus retain a high level of viability during drying and storage. The incorporation of such dried cultures into powdered milk may be the method of choice for preparing powdered milk products containing probiotics. However, research is needed including storage testing to confirm the feasibility of such a process.

Careful consideration should be given to factors such as the following, with respect to viability of the probiotic

- Drying method
- Type of packaging
- Size of packaging
- Storage conditions (temperature, humidity, etc.)
- Powder milk quality (Standard reference)
- Rehydration procedure
- Handling of rehydrated product

8.4. Prebiotics

Prebiotics as an area is distinct from probiotics and therefore, will not be covered in detail in this report. The Consultation recognizes both the potential benefits of prebiotics with respect to probiotics, in addition to their ability to stimulate indigenous beneficial bacteria in the host.

Prebiotics are generally defined as 'nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health' (Gibson and Roberfroid, 1995).

The concept of prebiotics essentially has the same aim as probiotics, which is to improve host health via modulation of the intestinal flora, although by a different mechanism. However, there are some cases in which prebiotics may be beneficial for the probiotic, especially with regard to bifidobacteria, that is the synbiotic concept. Synbiotics are defined as 'mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract of the host' (Andersson et al., 2001). If a synbiotic relationship is intended, then it should be verified scientifically, following the guidelines outlined in Section 5 of this report.

9. Post Market Surveillance

The Consultation recommends that probiotic producers, medical professionals and public health officers consider some form of system to monitor the health outcomes of long-term probiotic administration. This is suggested as a means to gain insight into potential side-effects as well as assess long-term benefits. A necessary prerequisite for surveillance is a proper trace-back system.

10. Conclusions

1. The experts agreed that adequate scientific evidence exists to indicate that there is potential for the derivation of health benefits from consuming food containing probiotics. However, it was felt that additional research data are needed to confirm a number of these health benefits in humans, applying a systematic approach and following the guidelines for the assessment of probiotics suggested in this report.

2. There is good evidence that specific strains of probiotics are safe for human use and able to confer some health benefits on the host, but such benefits cannot be extrapolated to other strains without experimentation.

3. The health benefits for which probiotics can be applied include conditions such as gastrointestinal infections, certain bowel disorders, allergy, and urogenital infections, which afflict a large portion of the world's population. The application of probiotics to prevent and treat these disorders should be more widely considered by the medical community.

4. In addition, there is emerging evidence to indicate that probiotics can be taken by otherwise healthy people as a means to prevent certain diseases and modulate host immunity.

5. The regulatory status of probiotics as a component in food is currently not established on an international basis. In only a few countries, regulatory procedures are in place or sufficiently developed to enable probiotic products to be allowed to describe specific health benefits.

11. Recommendations

1. Potential probiotic strains must be identified by methods including internationally accepted molecular techniques and named according to the International Code of Nomenclature, and strains should preferably be deposited in a reputable internationally recognized culture collection.

2. In order to be termed a probiotic, the probiotic microorganism must be able to confer defined health benefits on the host, as outlined in Section 5 of this Report, in the actual product vehicle that will be made available to humans.

3. There is a need for refinement of *in vitro* and *in vivo* tests to better predict the ability of probiotic microorganisms to function in humans.

4. There is a need for more statistically significant efficacy data in humans.

5. Good manufacturing practices must be applied with quality assurance, and shelf-life conditions established, and labelling made clear to include minimum dosage and verifiable health claims.

6. The regulatory status of probiotics as a component in food has to be established on an international level.

7. The Consultation recommends that a regulatory framework be established to better address issues related to probiotics including efficacy, safety, labelling, fraud and claims.

8. Probiotic products shown to confer defined health benefits on the host should be permitted to describe these specific health benefits.

9. Surveillance systems, including trace-back and post marketing surveillance, should be put in place to record and analyze any adverse events associated with probiotics in food. Such systems could also be used to monitor the long-term health benefits of probiotic strains.

10. Efforts should be made to make probiotic products more widely available, especially for relief work and populations at high risk of morbidity and mortality.

11. Further work is needed to address criteria and methodologies for probiotics.

12. List of Abbreviations

BV: Bacterial Vaginosis

CAC: Codex Alimentarius Commission

DNA: Deoxyribonucleic Acid

FAO: Food and Agriculture Organization of the United Nations

HACCP: Hazard Analysis Critical Control Point system

IgE: Immunoglobulin E

NK cells: natural killer cells

ORS: Oral Rehydration Salts

PFGE: Pulse Field Gel Electrophoresis

RDP: Ribosomal Database Project

RNA : Ribonucleic Acid

sIgA: secretory Immunoglobulin A

TH1: T helper lymphocytes 1

TH2: T helper lymphocytes 2

UTI: Urinary Tract Infection

WHO: World Health Organization

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