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PROBIOTICS – ARTICLE REVIEW

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Abstract:
Probiotics are used in a large variety of fields relevant to human and animal health. For some decades now, bacteria known as probiotics have been added to various foods because of their beneficial effects for human health. The number of products containing probiotics, viable bacteria with proven health benefits, entering the market is increasing. Traditionally, probiotics have been associated with gut health, and the oral consumption of probiotic microorganisms produces a protective effect on the gut flora. Lots of studies suggest that probiotics have beneficial effects on microbial disorders of the gut. They are known to have a positive therapeutic effect against traveller's diarrhoea, antibiotic associated diarrhoea and acute diarrhoea. Use of probiotics helps to protect the host from various intestinal disorders by increasing the number of beneficial bacteria. The most commonly used microorganisms in probiotic products are the lactic acid bacteria (LAB) and it is important to know how these LAB affect the immune status of the consumer. The discovery that probiotics can stimulate an immune response provides a scientific basis for some of the observed probiotic effects.

KEYWORDS: *Probiotics, lactic acid bacteria, gut flora, Classification and identification, MOA, Benefits.*

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INTRODUCTION:

PROBIOTICS DEFINITION

Probiotics are bacteria that help keep the natural balance of organisms (microflora) in the intestines. The normal human digestive tract contains about 400 types of probiotic bacteria that reduce the growth of harmful bacteria and promote a healthy digestive system. 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 American 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. 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.

What are the different types of probiotics?

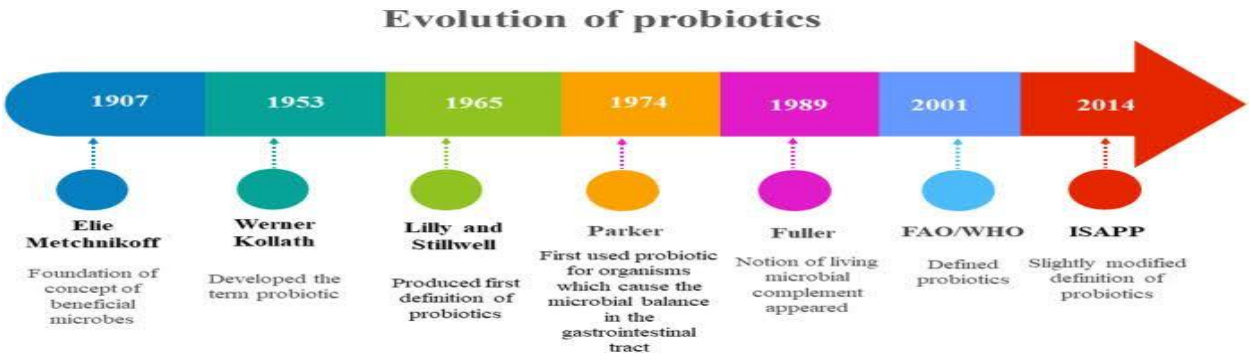
There are many types of bacteria classified as probiotics, but following two are the most commonly used bacteria as probiotics:

- Lactobacillus may be the most common probiotic. It's the one you'll find in yogurt and other fermented foods. Different strains can help with diarrhea and may help with people who can't digest lactose, the sugar in milk.
- Bifidobacterium can be found in some dairy products. It may help ease the symptoms of irritable bowel syndrome and some other conditions.
- Saccharomyces boulardii is a yeast found in probiotics. It appears to help fight diarrhea and other digestive problems.

HISTORY:

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 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 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 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 Havener 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).



MODERN PROBIOTICS:

In the 1990's, a new generation of scientists, armed with better technology and an appetite for new ideas, began researching the gut microbiome – the community of microbes living in our GI tract. They started to understand much more about how the microbes we eat interact with our bodies.

With more understanding came more respect for their potential, and more research dollars. In 2001, the World Health Organization (the ―WHO‖) at long last issued a formal definition of probiotics, kickstarting even more research. This led to a series of microbial discoveries that rocked the probiotic world.

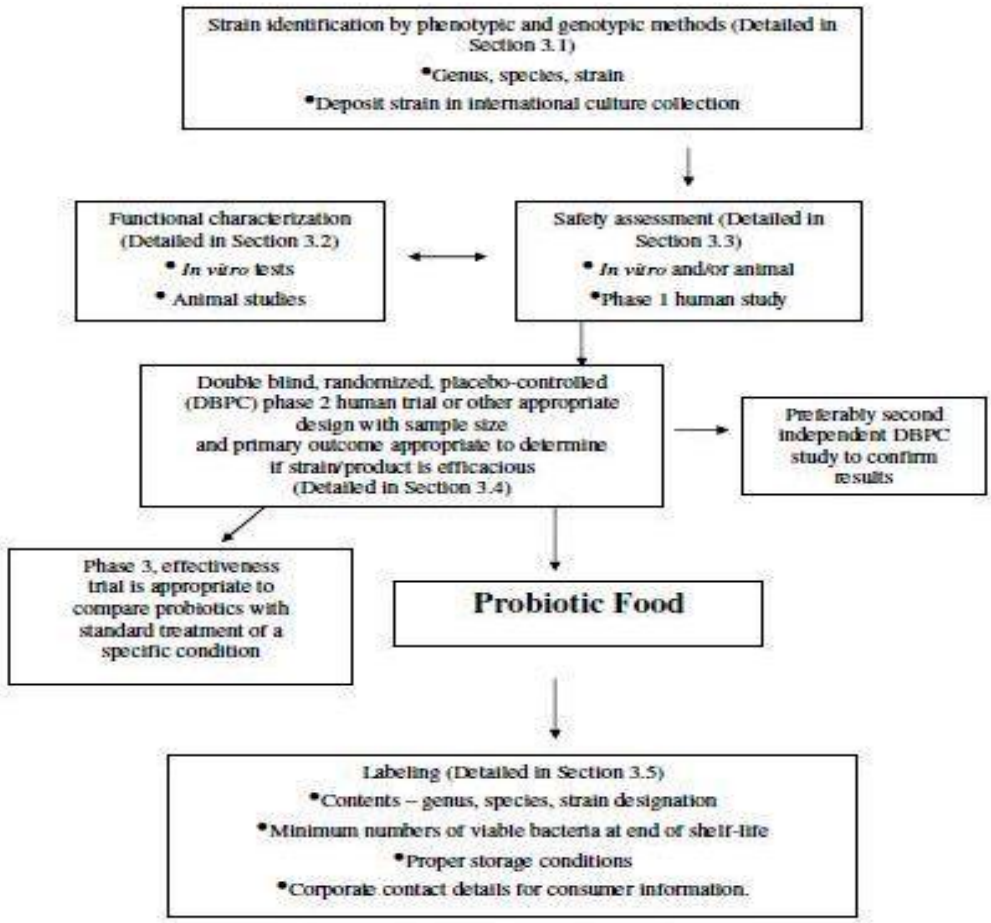
We learned how to more accurately identify microbial strains and to evaluate how each strain interacts with the body. We learned that many microbes used as probiotics don't even make it to the gut alive, but instead die when they encounter stomach acid, and that some microbes need extra ―food‖ in the form of prebiotic fiber to thrive. And we learned that too many of some species/strains of microbes in your gut actually isn't a good thing, but can instead be bad for your health.

GUIDELINES FOR ASSESSMENT OF PROBIOTICS:

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.

Figure 1. Guidelines for the Evaluation of Probiotics for Food Use



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. 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 micro flora 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.

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 cognized methods. For the latter, the RDP (ribosomal data base project) should be used to confirm identity.

IDENTIFICATION OF LACTOBACILLUS (LACTIC ACID BACTERIA):

Identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and oxidase tests, motility, indole producing, growth at 15°C, and carbohydrates fermentation test.

All Gram-positive and catalase-negative bacilli were selected for the assessment of antimicrobial ability. Antimicrobial effect of isolates was evaluated by disc diffusion test on MHA medium plated with three pathogens.

Growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed after incubation of all agar media at 37°C for 24 hours.

MECHANISM OF ACTION OF PRROBIOTICS:

The mode of action of probiotics involves their interaction with the gut microbiome and various physiological processes within the human body. Probiotics exert their beneficial effects through several mechanisms:

1. **Microbiome Balance**: Probiotics, when consumed, introduce beneficial bacteria into the gut microbiome. They compete with harmful microorganisms, such as pathogenic bacteria, for resources and adhesion sites in the intestinal lining. This competition can help maintain a balanced and diverse gut microbiome.
2. **Modulation of Immune Response**: Probiotics can influence the immune system by interacting with immune cells in the gut-associated lymphoid tissue. They can enhance the production of anti-inflammatory compounds while reducing the production of pro-inflammatory molecules, helping to regulate the immune response.
3. **Production of Bioactive Compounds**: Some probiotic strains produce bioactive compounds, such as short-chain fatty acids (SCFAs) and antimicrobial peptides. SCFAs can have anti-inflammatory effects and promote the health of intestinal cells, while antimicrobial peptides can inhibit the growth of harmful bacteria.
4. **Enhancement of Gut Barrier Function**: Probiotics can help strengthen the intestinal barrier, preventing the passage of harmful substances and pathogens into the bloodstream. This may reduce the risk of leaky gut syndrome and associated health issues.
5. **Metabolism of Dietary Compounds**: Probiotic bacteria can metabolize dietary components, including fibres and complex carbohydrates, into bioactive molecules like vitamins and other nutrients. This can improve nutrient absorption and promote overall health.
6. **Reduction of Toxin Production:** Some probiotics can reduce the production of toxins by pathogenic bacteria in the gut. This can be particularly beneficial in preventing foodborne illnesses and gastrointestinal infections.
7. **Synthesis of Beneficial Compounds:** Certain probiotic strains are capable of producing substances like vitamins (e.g., B vitamins) and

enzymes (e.g., lactase) that can support overall health and digestion.

8. **Neurotransmitter Production:** Emerging research suggests that the gut microbiome, influenced by probiotics, may play a role in the production of neurotransmitters like serotonin. This connection between gut health and mental health is an area of ongoing investigation.

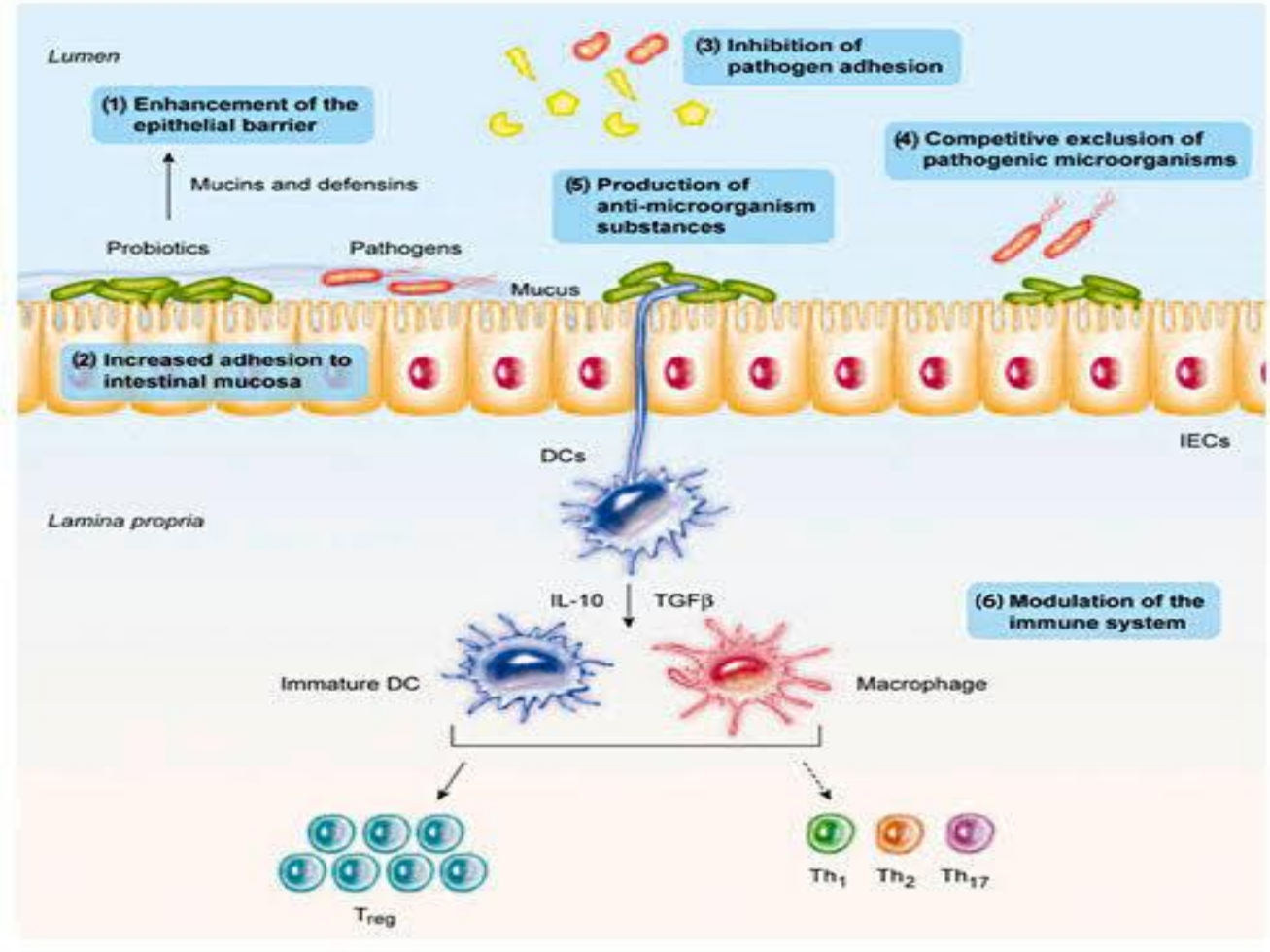


FIG : MOA OF PROBIOTICS

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.

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. It would be preferable to have such findings corroborated by more than one independent centre.

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, little is known about the molecular mechanisms of the benefits reported. 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 enzymes or metabolites that act directly on the microorganism 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. These traditional starters are not considered probiotics since they lack the ability to proliferate in the intestine.

SAFETY CONSIDERATIONS:

Antimicrobial resistance profiles of probiotics

As with any bacteria, antibiotic resistance exists among some lactic acid bacteria, including probiotic microorganisms. 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 mobilized 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.

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. 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. 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.

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 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.
B. Certain strains of vancomycin resistant enterococci are commonly associated with nosocomial infections in hospitals. 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.

PROBIOTIC PRODUCT SPECIFICATIONS, QUALITY ASSURANCE AND 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. 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.

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. 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 and environmental adaptation. Probiotic stability during powder storage is inversely related to storage temperature 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

ADVANTAGES:

Preliminary research suggests that probiotics may provide some benefit to those who suffer from irritable bowel syndrome.

Prevention of diarrhoea caused by certain pathogenic bacteria and viruses:

Infectious diarrhoea 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 diarrhoea 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 and treatment of acute diarrhoea 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 entero pathogens and animal studies have indicated beneficial effects against pathogens such as Salmonella. There is evidence from studies on travellers' diarrhoea, where some of the causative pathogens have been presumed to be bacterial in nature, that benefits can accrue with probiotic administration.

It is important to note that probiotic therapy of acute diarrhoea should be combined with rehydration if available.

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. The intestinal micro flora likely plays a critical role in inflammatory conditions in the gut, and potentially probiotics could remediate such conditions through modulation of the micro flora. Clinical and mechanistic studies are urgently required to better understand the interface between the microbes, host cells, mucus and immune defences, and to create efficacious interventions. Such studies should include molecular examination of the intestinal (not only faecal) flora and long-term (5-10 years) effects of probiotic microorganisms.

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 micro flora 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. 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. 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 Propioni bacterium sp. showed a decrease in availability of carcinogenic aflatoxin in the lumen. However, it is too early to make definitive clinical conclusions regarding the efficacy of probiotics in cancer prevention.

Cardiovascular disease

There is preliminary evidence that use of probiotic lactobacilli and metabolic byproducts
potentially confer benefits to the heart, including prevention and therapy of various ischemic heart syndromes 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.

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.

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. 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. 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.

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