

**Report from the Definitions and Standards
Working Group
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Justification for the value of probiotics/prebiotics - societal human impact

Probiotics and prebiotics may be helpful for providing a layer of protection for prevention of various types of infections. This could not only reduce suffering associated with morbidity (and perhaps even mortality), but also decrease the clinical use of antibiotics, thereby furthering efforts to slow the spread of antibiotic resistant pathogens. In a climate of soaring incidence rates of allergy, emerging evidence suggests that early administration of probiotics may prevent allergy development in susceptible populations. Probiotics may also provide a therapeutic approach to treatment of some bowel disorders, such as irritable bowel disease (IBD) and irritable bowel syndrome (IBS). This is especially important where standard therapies are either extreme (surgical intervention) or not suitably effective.

The overall objective of the ISAPP Working Group is to identify issues important to the advancement of the science of probiotics and prebiotics by promoting the interdisciplinary interactions among scientists in the field and to raise awareness among the public, health professionals and regulatory officials. The Definitions and Standards sub-group supported this effort by focusing on definitions and standards for probiotics and prebiotics and their use in commercial products.

Current State of Affairs for Definitions and Standards

The marketing of probiotic products has occurred concurrent with the development of the science substantiating use of such products. Furthermore there is little or no enforced regulation worldwide regarding the quality or efficacy of probiotic products. In the USA, a few probiotic products have been removed from the market because of non-allowable disease claims. But products mislabeled with regard to genus and species, cell count and/or unsubstantiated structure/function statements remain on the market. This mislabeling of products is not unique to the USA and if continued could result in consumer disillusionment and a subsequent declining market.

The term 'probiotic' remains undefined legally in many countries, although there are several concurrent efforts to propose a more universal definition. Although the term implies a health

benefit to the consumer, it can be used on a label even if the product has no substantiation of a beneficial health effect on humans. Therefore, there is a need to rectify this situation to allow consumers to distinguish products based on quality standards. An opportunity exists for a science-driven approach to clarify what probiotics and prebiotics are and to make recommendations to improve their commercial use. The benefit of this effort will be the improvement of the products on the market which hopefully will translate into a better impact on human health.

Regulatory approaches to probiotics and prebiotics differ among different countries worldwide. There is no harmonization of standards among EU countries. Diverse categories encompass probiotic products, including: food, functional food, novel food, natural remedy (Denmark Sweden and Finland), natural health product (Canada), dietetic food (Italy), dietary supplement (USA), biotherapeutic and pharmaceuticals (probiotic pharmaceuticals available in Canada, China, eastern European countries, France, Germany, Belgium, Austria, Italy). Groups with established or currently evolving definitions of probiotics include:

- Canada's Natural Health Products Directorate – in progress
- European Food & Feed Culture Association (EFFCA - an industry association) – draft in progress
- FAO/WHO – proposed definition with guidelines for their use in food (FAO/WHO, 2001 and 2002)
- FDA – in progress

The scope of the Natural Health Products Directorate initiative in Canada includes vitamins, amino acids, minerals, herbals, extracts and probiotics. In this initiative, probiotic was defined as “monoculture or mixed culture of live microorganisms that benefit the microflora indigenous to humans” (Canadian Gazette part 1, Dec. 22, 2001). Allowable claims on Natural Health Products as defined for Canadian regulations are based on ‘levels of evidence’ (Levels I-IV), ranging from well-designed meta-analysis of randomized controlled multicenter trials to traditional references. The initiative moving forward requires pre-market approval for Natural Health Products. FAO/WHO efforts are currently focused on establishing guidelines for the use of the term ‘probiotic’ in foods and levels of evidence necessary to make a health claim. Their recommendations will be considered by applicable Codex committees, including labeling (which deals with claims); nutrition and foods for special dietary uses; and hygiene. As Codex is concerned only with food, no consideration was given by this group to probiotics used in pharmaceuticals or dietary supplements. The definition proposed by an Expert Consultation convened by FAO/WHO (2001) is: ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host’. In the United States, probiotics for human use can be used in several legally defined classes of products, including foods, dietary supplements and drugs, all of which fall under the regulatory realm of the FDA. There is no official definition of probiotic in Japanese regulation, but several probiotic and prebiotic products have achieved FOSHU (Foods for Specialized Health Use) status and health statements have been approved by the Japanese Ministry of Health. EFFCA, members of Lactic Acid Bacteria Industrial Platform (LABIP), accepted the LABIP conclusion of 1998 [“For the demonstration of a specified probiotic (functional health) activity of a certain strain, well designed human studies (double blinded, placebo controlled, sufficient representative numbers of probiotics) are required, and the proof of efficacy in humans should be granted by at least one well-designed human study”]. These diverse groups interested in probiotics are converging on the same conclusion: that use of the term “probiotic” should refer only to products demonstrating beneficial functional or health activities substantiated by human efficacy studies.

It is interesting that although definitions and standards for probiotics for human use are not developed in many countries, probiotic-containing feed additives are highly regulated in Europe, USA and Canada, with requirements of pre-market approval, safety assessment, geographically specific efficacy studies, requirements for strain deposit, standards for acceptable contamination limits. In contrast, there are no specific regulations for starter cultures or probiotics for human use.

Where are the gaps and how to fill the gaps:

Definitions. There are many published definitions of the term “probiotic”, which differ with regard to several attributes including specification of mechanism of action, route of administration (oral vs. other methods of application) and the requirement of viability. In an effort to harmonize understanding of the term with consideration of the current state of the science, a definition of probiotics was proposed as “defined, live microorganisms administered in adequate amounts which confer a beneficial physiological effect on the host”. This definition is very similar to that proposed by Guarner and Schaafsma (1998) but includes non-oral applications. The scope of this definition is quite inclusive, however, and would conceivably include microbes such as those used as live vaccines, biologics or therapeutic bacteriophage. Therefore, some exclusions for this definition should be considered. This definition requires that the term ‘probiotic’ only be applied to defined microbes having a substantiated beneficial physiological effect. Another implication is that live microbes used as delivery vehicles for physiologically beneficial components to the gastrointestinal (GI) tract would be probiotic even if they did not survive intestinal transit. Therefore, strains of the species *S. thermophilus*, for example, would be considered probiotic. Although non-viable bacteria may mediate a physiological benefit, they are not considered to be ‘probiotics’ under this definition. Another term should be defined to encompass such products, such as nonabiotic or abiotic. The term ‘beneficial physiological effect’ is inclusive of both health effects and functional effects. Functional effects would include endpoints such as quality of life indices, fecal microecology, cholesterol lowering, immune enhancement indicators and metabolic markers. It is noteworthy that Codex usage of the term ‘health claim’ includes nutrient function, enhanced function and reduction of disease, therefore inclusive of physiological benefits (such as reduction of cholesterol levels or improved immune markers) that may be indicative of a health effect.

A prebiotic was defined as “a non-digestible substance that provides a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria”. This definition may overlap significantly with the definition of dietary fiber (a whole food) or ‘added fiber’. The Food and Nutrition Board of the Institute of Medicine of the National Academy of Sciences proposed definitions for fibers in March 2001-as follows:

- Dietary Fiber consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants
- Added Fiber consists of isolated, nondigestible carbohydrates that have beneficial physiological effects in humans
- Total Fiber is the sum of Dietary Fiber and Added Fiber

Prebiotic compounds such as resistant starch and inulin, which do not qualify as dietary fiber with current AOAC methods, could be considered Added Fiber if they show beneficial physiological effects in humans. Under consideration by the FDA is the proposition that fiber added to a food must show a demonstrated physiological benefit.

It is perhaps noteworthy that a very different definition of ‘prebiotic’ can also be found in scientific usage. The Dorland 29ed Medical Dictionary defines prebiotic as the “period before the existence of life on earth”.

A synbiotic was defined as “a product that contains both probiotics and prebiotics”. An implication of this definition is that demonstration of a synergistic effect is not a requirement of synbiotics.

Labeling. From a scientific perspective, the suitable description of a probiotic product should include:

- Genus
- Species
- Strain designation
- Viable count of each strain at end of shelf life
- Storage conditions
- Dose should be specified and should relate to the levels tested documenting the physiological effect
- An accurate description of the physiological effect, as allowable by law
- Contact information for post-market surveillance

Strains should be identified with nomenclature consistent with current scientifically recognized names. The most recently validated names can be retrieved from www.bacterio.cict.fr/ and are published in the International Journal of Systematic Bacteriology. A list of approved bacterial names was published in 1980 in the Int. J. Syst. Bacteriol (30:225-420). DNA-based techniques are essential for determining correct speciation of probiotic strains. Although DNA-DNA homology with established type strains is the preferred method for speciation, 16S rDNA sequencing combined with phenotypic analysis is also suitable.

Additionally, all strains should be identified with a strain designation. This is important for strain documentation, tracking, product consistency and quality control, post-market surveillance and all research efforts, including human studies. Furthermore, as the current state of evidence supports the strain dependency of functional effects, strain identification is essential. In addition, all commercial probiotic strains should be deposited in a collection recognized by the International Depository Authority.

Labeling of prebiotics should also include information on source and specific dose of active component based on levels documenting the selective impact on certain indigenous bacteria or physiological benefit on the host.

Dose. With probiotics, there are very few studies which establish the minimum effective dose for a physiological effect. Reviewing the published literature on positive physiological benefits in humans, a general trend emerges that positive results are rarely seen at daily doses less than 10^{9-10} cfu. Often, this is the minimum dose used in studies, but studies with negative results have been published which tested low administration levels. The effective administered dose will likely vary with probiotic strain, test subject, delivery form and if taken with food. However, general conclusions on the impact of these variables on dose cannot be made, as they have not been systematically studied. Stabilization or encapsulation advances may lead to the protection of cells through the GI tract resulting in delivery of cells to the target site. Such stabilization technology may ultimately lead to the ability to reduce the intake of viable cells, since fewer are killed during transit. Missing are studies which report the effective dose at the target site rather than at the point of administration.

The effective dose of prebiotics seems to be about 1-3 g/d for infants and 5-15 g/d for adults. Effective dose differs for different prebiotics. As prebiotics are generally mixtures of different chain length carbohydrates, the dose of the active components is difficult to determine. Additional studies are needed to define both the effective dose levels and maximum tolerated dose for individual prebiotics.

Requirements for substantiation of efficacy. An FAO/WHO Working Group recommended that physiological benefit of probiotic foods be substantiated with a phase 2, double blind, randomized, placebo-controlled trial (FAO/WHO, 2002). (In some cases a placebo-controlled trial is not possible. If, for example, an endpoint is chosen where an established therapy exists, placebo-controlled studies cannot be conducted and a different design would be necessary.) Clearly, human studies are essential before a physiological benefit for humans is claimed. The strength of the claim should be correlated with the level of evidence. The difficulty of identifying health benefits in healthy persons suggests the value of functional markers for substantiating probiotic effects. Canadian efforts to establish standards for evidence for evaluating foods with health claims is found at http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_summary_report_symposium_consultation.html.

Standards for Good Clinical Practice have been delineated by the International Committee on Harmonization (www.ifpma.org/ich1.html). Although these standards are designed for pharmaceutical development, they can be applied to human research on probiotics.

Requirements for substantiation of safety. Relatively few microbes have been tested in safety and tolerance studies. Tolerance tests for species of *Lactobacillus rhamnosus*, *helveticus*, *bulgaricus* and *Bifidobacterium longum* have shown that these bacteria are tolerated at levels greater than 6 g/kg body weight (Donohue, et al., 1993). *L. rhamnosus*, *L. acidophilus* and *Bifidobacterium lactis* were tested in tolerance studies and shown to be safe at 50 g/kg/d for a mouse, which extrapolated to 35 g/d for a 70 kg person (Zhou, et al. 2000). There is no indication that lactobacilli or bifidobacteria given to a healthy host presents a health risk (Salminen, et al., 1998). In patients with underlying disease, commensal lactobacilli have been associated with endocarditis and septicemia but only in very rare cases. Endocarditis due to lactobacilli were estimated at 0.11 cases/million (Gasser, 1994). Lactobacilli were associated with septicemia at a level of 0.102% measured over 3 years (Gasser, 1994). The most frequent species associated with these *Lactobacillus* infections were *rhamnosus*, *casei*, *paracasei*, and *plantarum*. All cases were attributed to commensal lactobacilli. Probiotic lactobacilli and bifidobacteria have been used in food products and dietary supplements for decades, with a compelling record for safe consumption. When considering the use of enterococci as probiotics, the situation is more complicated. Although *Enterococcus* strains have some history of safe use as probiotics and food starter cultures, Franz, et al. (1999) concluded that in the past enterococci were considered "...harmless commensals with low pathogenic potential" but today "...may be considered opportunistic pathogens". They acknowledge that the link between food sources of *Enterococcus* and infection is not established, but it must be considered that the surveillance of these products is not extensive. The definition of probiotic proposed here is not limited to the use of lactic acid bacteria. In fact, strains of *E. coli*, *Bacillus* and *Saccharomyces* have been studied for probiotic effects, and are being marketed as biotherapeutic probiotics.

Pregnancy and life age extremes are not opportunistic factors and consumption of therapeutic products containing live lactic acid bacteria or fermented products by healthy subjects has never led to proven published cases of infection. Lactic acid bacteria can behave opportunistically but

extremely rarely. Regardless of infection types, incidence rate is so low it is impossible to determine with accuracy (Gasser, personal communication).

Considering the range of probiotic products possible within the definition of probiotics offered here, the use of probiotics as biotherapeutics or drugs must be considered, as these products are targeted for people with underlying health conditions. Very rare complications have been reported:

- D-lactic acidosis when short bowel syndrome is present (Day and Abbott, 1999)
- Liver abscess caused by *L. rhamnosus* (Rautio, et al. 1999)
 - 74-year old hypertensive, diabetic woman
 - Consumed 500 ml GG drink/day
 - Strain isolated from infection indistinguishable from GG; same DNA finger print from strain from infant with no exposure to GG products
- Endocarditis caused by *L. rhamnosus* (Mackay et al. 1999)
 - 67-year old, history of mitral valve prolapse and tooth removal
 - Chewed dried, mixed-strain probiotic preparation, containing *L. rhamnosus*, *E. faecalis*, *L. acidophilus* and others
 - Strain isolated from infection indistinguishable from strain in probiotic supplement
- Thirteen cases of *Saccharomyces* fungemia due to vascular catheter contamination (Hennequin et al., 2000).
- *Bacillus* infections linked to probiotic consumption include three reports (Spinosa et al., 2000; Oggioni et al., 1998; Richard et al., 1988) detailing seven cases of *B. subtilis* bacteremia, septicemia and cholangitis, all in patients with underlying disease.
- No cases of infections from *Bifidobacterium* have been reported.

Considerations for safety should include history of safe use under the recommended route of administration, frequency of association of species with infection, likelihood of production of deleterious metabolic end-products, association with transferable antibiotic resistance, sensitivity to therapeutic antibiotics and relatedness to species which produce hemolysins or mammalian toxins. The lessons about safety from pathogenic bacteria are not applicable to non-pathogenic lactobacilli or bifidobacteria.

It is noteworthy that in May 2001, the FDA Center for Food Safety and Applied Nutrition indicated as one of 20 priority research needs to support its regulatory mission included developing methods to assess the safety of bacteria used as probiotics. Such studies should include the evaluation of health effects, mechanism of action and stability characteristics of the specific strain (or strain combinations), the definition of physiologically relevant consumption levels, and the definition of the active principle (whole cell, viable / nonviable cell, cell components) of a probiotic product.

List of appropriate species to be used as probiotics. The question of whether or not a list of species appropriate for probiotic use was considered. Presumably, such a list would be based on evaluation of safety and efficacy data for different probiotic species and would provide guidance to manufacturers on choice of species for commercial products.

There is some precedent for lists of approved microorganisms. The FDA does list some bacteria in Federal Register 21 CFR Parts 131, 133, 136 and 137 that may be contained in or be derived from microorganisms. This includes *L. bulgaricus*, *S. thermophilus*, *L. acidophilus* and 'lactic acid producing bacterial culture', but this list is not meant to be a complete listing. Not on the list would be ingredients that companies self affirm as 'generally recognized as safe' based on a

comprehensive review of safety factors. (In this case, documents are not necessarily filed with the FDA and therefore are not part of the public record.)

In Europe, microorganisms with no traditional use in food production are considered 'novel food' and fall under Regulation (EC) No 258/97. This regulation theoretically establishes a 'list of approved probiotics for novel foods'. However, to date, no such applications or assessments have been made.

The International Dairy Federation (IDF) prepared a list of microorganisms with a documented history of use in food. These microbes were documented to be used in foods before 1997 and may provide precedent for an EU 'GRAS list' for microbes. This list, developed in conjunction with EFFCA, was published in the IDF Bulletin (377/2002). In Canada, according to the 'Labeling Standard' for 'Intestinal Flora Modifiers' for a pharmaceutical product (not a food), only *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *L. casei*, *L. helveticus*, *B. bifidum*, *B. longum*, *B. breve* and *S. thermophilus* can be used if a claim relating to 'GI tract restoration' is made. If no claim is made, microorganisms other than those listed can be used. If a company desires to label a product containing a bacterium not on the list with the claim, a full drug application would have to be filed.

A list delineating species appropriate as probiotics would ignore the importance of intended use, e.g., target population, route of administration, strain-specific attributes and other criteria that may impact safe use. Furthermore, such a list could be unnecessarily limiting and bureaucratically untenable. It was deemed more practical that a case for safety and efficacy be made for each probiotic strain, following the recommendations made above rather than to develop a list of approved probiotic species. This is especially important as many probiotic attributes are strain-specific, not species-specific.

Recommendations to editors on key elements that are advised to be included in probiotic/prebiotic publications

- Publication of both positive and negative results, especially clinical studies
- Require identification of strain with appropriately validated genetic and phenotypic technologies and disclosure of such in publication
- Verification of probiotic viability before, during and at end of trial
- Accurate description of the source and dose of prebiotic ingredients
- Request information that relates probiotic colonization with physiologic benefit
- Document how and when probiotic was taken (with food, in am or pm, etc)
- List any concurrent medications
- Document compliance

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