

IPA guidelines to qualify a microorganism to be termed as 'probiotic'

This guide's intent is to represent current practices and scientific principles adopted by probiotic manufacturers/strain-owners worldwide to qualify a microorganism to be termed as probiotic. Additional analysis/ procedures may be required to fulfil specific regulatory requirements depending on particular jurisdictions and/or on the role of the party that is involved (e.g. contract manufacturer, distributor, etc.)

In 2001, in a period that marked a rise in interest in probiotics for the clinical and scientific communities, experts convened by the FAO/WHO provided a scientific opinion on "probiotics"¹ and agreed on the following definition (later amended by an expert consensus group): "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*"^{2,3}.

This report was followed by the "Guidelines for the Evaluation of Probiotics in Food"⁴ where the FAO/WHO experts made several recommendations. One of these was to adopt the definition as well as more specific criteria as a prerequisite to qualify a microbial strain as a "probiotic".

While the definition of probiotics has been widely acknowledged by the scientific community and key players in the field of probiotics, the FAO/WHO guidelines have not been globally implemented. In many parts of the World, there is neither regulatory status nor guidelines defining the probiotics category, nor a commonly acknowledged list of individual probiotic strains and/or species⁵.

Therefore, it is essential that the industry clarifies recommendations which ensure the proper use of the term "probiotic(s)" without contradicting national requirements. It is imperative to distinguish probiotics from the more general category of *live microbial cultures*. Probiotics are not generally added to foods to provide a technological function, and are not used as starter cultures for yogurt- or cheese-making. While some starter cultures can also exhibit probiotic properties, and can be used as such, the category of *probiotics* should be separated from the broader category of live microbial cultures, as probiotics comprise a niche group recognized as exhibiting certain characteristics that promote survival in the human gastrointestinal tract, and provide a health benefit to the host. Furthermore, despite the fact that to date the most widely recognized probiotics are strains of lactic acid bacteria, this category should not be limited, as some Gram (+) spore formers and even strains of yeast have been demonstrated to promote health benefits when consumed in adequate doses. Distinguishing probiotics from the broader category of live microbial cultures allows us to move forward to further define the characteristics and properties of probiotics.

¹ 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 and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid bacteria. Cordoba, Argentina, 1 - 4 October 2001.

² Note: but restricted its scope to discussion of 'Live microorganisms which when consumed in adequate amounts as part of food confer a health benefit on the host.'

³ The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Hill C *et al* (2014) *Nature Rev Gastroenterol Hepatol* **11**:506-514.

⁴ Joint FAO/WHO Working Group Report on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada. 30 April – 1 May 2002.

⁵ Note: Some countries have adopted such lists and others have developed certain conditions for qualifying specific strains as probiotic.

⁶ Health Canada (2015). Natural Health Products – Probiotics. <http://webprod.hc-sc.gc.ca/nhp/nd-bdipsn/atReq.do?atid=probio>

⁷ Ministry of Health (2013). Guidelines on probiotics and prebiotics.

http://www.salute.gov.it/imgs/C_17_pubblicazioni_1016_ulterioriallegati_ulterioreallegato_0_alleg.pdf

Scope of the IPA guidelines

In accordance with the FAO/WHO guidelines, the scope of this document is limited to the use of probiotics in food, including food supplements (Chapter 3¹). Drug applications and animal feeds are excluded from the scope of these guidelines.

Probiotic criteria

IPA recommends that, with respect to commercial communications, the term ‘probiotic’ should only be used to describe microorganisms when a certain combination of requirements is met. No specific authorisation would be required as long as no reference to any specific health effect is made outside of structure/function statements that indicate an effect of the probiotic on the normal structure and/or function of the body, when the regulatory framework allows for it. For example, in accordance with Italian⁶ and Canadian⁷ guidelines, IPA considers that microorganisms described as ‘probiotic’ and meeting the established conditions, facilitate a beneficial balance of the intestinal microbiota and/or support desirable gastrointestinal function leading to beneficial effects in humans.

As the intention of this document is to provide information that should be considered on a global scale when identifying a microorganism as probiotic, the following steps are recommended as inclusive criteria for the category of probiotics as understood to date. We recommend consideration of Genus and species initially, however because it is well documented that many probiotic characteristics and health benefits are strain specific, we also recommend specific identification to the strain level. The areas of probiotic characteristics and safety should be considered as well. Please consider the following as criteria that should be considered in categorizing a microorganism as a probiotic that can be regarded as safe for use in its intended application.

Probiotic strains:

Taxonomy must be determined at Genus and species level Probiotics are specific strains that need to be definitively identified by using current, valid and internationally accepted techniques. This may require using a combination of the most appropriate molecular techniques, with species designation indicated according, but not limited to, the LPSN list (List of Prokaryotic Names with Standing in Nomenclature <http://www.bacterio.net/>)⁶, the German Collection of Microorganisms and Cell Cultures (<https://www.dsmz.de/>), or International Committee on Systematics of Prokaryotes (<http://icsp.org/>).

With the advent of whole genome sequencing, the capability to accurately identify and characterize probiotics is readily available. This in-depth discovery tool can be used to avoid the use of noncomprehensive methods, such as 16S rRNA sequencing, as the sole identification method for probiotic bacteria. The investigation at the level of the whole genome allows alignment of the probiotic strain of interest with similar strains that have potentially been well-studied and characterized. Alignment of the nucleotide sequence allows proper identification to the most similar Genus and species, and it also allows the level of differentiation to be understood between strains.

Probiotics should be identifiable at the strain level

As probiotics within the same Genus and species can have strain-specific and unique characteristics, it is imperative to identify probiotics to the strain level. Differentiation between strains within a species allows proper identification in addition to the discrimination of probiotics strains as specific/distinct ingredients. This level of identification also ensures that the correct strains are housed within internal culture collections, processed accordingly, and incorporated into the appropriate product. Whole genome sequencing, restriction fragment length polymorphism (RFLP), MLST and Random Amplified Polymorphic DNA (RAPD-PCR) are some of the methods that can be used to identify unique stretches of DNA that can

⁶ See also http://ijs.sgmjournals.org/content/64/Pt_4/1434.full.

be targeted in strain specific detection methods. These methods can be employed to identify and distinguish probiotic strains from those that are closely related.

This principle is in agreement with emerging requirements from authorities worldwide to ensure appropriate identification of components within the food/dietary supplement. Methods used for appropriate strain-specific identification and characterization may be shared between interested parties when required/needed. It is recommended that probiotic strains are deposited in an internationally recognised culture collection (wfcc.info/collections).

Probiotic strains should be characterized

Probiotics were originally described as microorganisms that promote the growth of other microorganisms. This description has been expanded to indicate that promotion of microbial growth specifically provides a beneficial balance of autochthonous microbial populations within the gastrointestinal tract (Holzapfel et al., 2001). While there are a number of traits that probiotics are believed to possess, defining a list specific to probiotics is troublesome, as not all probiotics produce the same metabolites or exhibit similar phenotypic traits. However, at a minimum, it is understood that the probiotic strain of interest should be able to survive and enhance the presence of beneficial microbes within the host.

Acid resistance and bile salt tolerance are two of the most distinguishing traits that identify strains as potential probiotic candidates, as these basic characteristics allow the strain to remain viable and survive passage through the harsh environment of the digestive tract. In addition, probiotic strains may promote colonization by adhering to microvilli, produce metabolites that inhibit the growth of pathogens, and exhibit certain metabolic capabilities such as production of bile salt hydrolase and vitamins.

Documented History of Safe Use The intended use of a probiotic determines its classification, such as but not limited to, an ingredient in a food or food supplement, a natural health product, a biological agent or a drug. In addition to this classification by intended use, the target consumer and appropriate dose must also be taken into consideration. There are a number of requirements that have been put together by regulatory agencies around the world to establish the safety of probiotics. See appendix for more details.

In Summary, as a variety of regional positions together define the global perspective of probiotics, the overwhelming commonalities that comprise the definition include viability, and evidence of health promoting properties. Regional regulations for commercialization of probiotics may vary, however safety and efficacy must always be proven, with evidence according to the level of the claim to be made.

It is expected that the global view of probiotics of today may change over time, however the aspect of health-promoting microorganisms should prevail within the definition. As a practice, microorganisms that can be used to supplement the diet for the promotion of health should be established as safe, and should be identifiable and distinguishable from similar microorganisms. Despite the scope of intended use, health-promotion is a key characteristic of this unique group of live microorganisms.

APPENDIX

⁷The European Food Safety Authority (EFSA) has taken responsibility to launch the Qualified Presumption of Safety (QPS) initiative in Europe.^{7,8} QPS aims to allow species that have historically been used as live microbial food cultures, are safe for human consumption and have an established history and safety status to be freed from the need for further safety assessment (other than satisfying any qualifications specified, like the assessment of antibiotic resistance) in the corresponding market authorisation procedures as sources of food/food supplements. The QPS concept is a fast track approach for species with a sufficient

⁷ <http://www.efsa.europa.eu/en/topics/topic/qps.htm?wtr=01>

⁸ Anon (2005). European Food Safety Authority (EFSA) Scientific Colloquium—Microorganisms in Food and Feed: Qualified Presumption of Safety. 13–14 December 2004, Brussels, Belgium. <http://www.efsa.europa.eu/en/supporting/pub/109e.htm>.

body of historical knowledge, so that all strains within those species listed are presumed to be safe for human consumption once qualification conditions are met. Antibiotic resistance is assessed and deemed acceptable.

A similar list has been put together by the International Dairy Federation (IDF). A number of live microbial cultures with a safe history of use are also listed in the IDF Bulletin No. 377: *Inventory of Microorganisms with a Documented History of Use in Food* (Mogensen G, 2002), which includes most of those listed by EFSA in the QPS list. A more recent IDF Bulletin No. 455, *Safety Demonstration of Microbial Food Cultures in Fermented Food Products*, provides an update to the aforementioned inventory of microbial species, taking a global perspective versus the original focus of European dairy products. This list should also be considered when investigating a safe history of use of probiotic strains.

In the United States, the U.S. Food and Drug Administration (U.S. FDA) has partially addressed the safety of microorganisms, including probiotics, used in foods and dietary supplements in their GRAS (Generally Recognized as Safe) and NDI (New Dietary Ingredients) guidance documents, respectively.

Under sections 201(s) and 409 of the Federal Food, Drug, and Cosmetic Act (the Act), any substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excluded from the definition of a food additive. Under sections 201(s) and 409 of the Act, and FDA's implementing regulations in 21 CFR 170.3 and 21 CFR 170.30, the use of a food substance may be GRAS either through scientific procedures or, for a substance used in food before 1958, through experience based on common use in food. (<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/>)

Although it is on voluntary basis, the U.S. FDA has received GRAS notifications for a number of probiotic microorganisms, where scientific evidence has been presented to establish the safety of these probiotic strains for use in food at specific, established doses (usually in colony forming units per volume). FDA had no questions.

Furthermore, the aspect of safety has been addressed within the category of "dietary ingredient" in the U.S., as stated in the NDI Draft Guidance document. U.S. FDA also states that a bacterial microorganism is a dietary ingredient if it is a dietary substance (an intentional constituent of food) or otherwise falls within one of the dietary ingredient categories listed in 21 U.S.C. 321(ff)(1). For example, bacteria that are used to produce fermented foods that are eaten without a cooking or pasteurization step (e.g., lactic acid bacteria used to produce cheese or yogurt) could be "dietary substances for use by man to supplement the diet by increasing the total dietary intake," which are defined as dietary ingredients in section 201(ff)(1)(E) of the FD&C Act (21 U.S.C. 321(ff)(1)(E)). FDA does not have a separate regulatory category or definition for dietary ingredients consisting of live or viable microorganisms.

(<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm257563.htm>)

While U.S. FDA does not have a complete list of acceptable microorganisms/probiotics for use in foods and dietary supplements, they do have the Partial List Of Microorganisms And Microbial-Derived Ingredients That Are Used In Foods; U. S. Food and Drug Administration Center for Food Safety & Applied Nutrition Office of Food Additive Safety of July 2001, in which they state, "Prior sanctions were granted for the use of **harmless** lactic acid producing bacteria, such as *Lactobacillus acidophilus*, as optional ingredients in specified standardized foods. These bacteria are permitted for use in cultured milk (which includes buttermilk) (§ 131.12), sour cream (§ 131.160), cottage cheese (§ 133.128), and yogurt (§ 131.200), provided that the mandatory cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are also used in the yogurt."

Probiotics should be distinguished from live microbial cultures although many of the same criteria are used to establish safety and are considered relevant in demonstrating a safe history of use. A recent publication (Pariza et al., 2015) outlines a number of criteria that should be considered when determining the safety of live microbial cultures, including probiotics. This decision tree includes the general traits that must be considered when determining safety and provides a step-by-step approach to proving safety. When combined with regulatory requirements, demonstrating these relevant aspects of safety to the strain level provides an assurance of safety beyond that of any species list with a general presumption of safety.

Identification and characterization at the strain level provide a thorough understanding of each strain and provide a traceability aspect of the specific strain proven to promote health benefits or certain unique characteristics for inclusion in a product.

Health Canada provides a unique approach to addressing the safety of probiotics by specifically listing requirements in the Natural Health Products Probiotics Monograph. This monograph is intended to serve as a guide to industry for the preparation of Product License Applications (PLAs) and labels for natural health product market authorization. Upon submission of a PLA, the applicant is attesting that the product will comply fully with the recommended conditions of use and specifications section outlined in this monograph. These include species identification, strain characterization, quantification in colony forming units (CFU), and a complete assessment of virulence properties (including but not limited to: antibiotic resistance profile, virulence factor production, and toxigenic activity). A list of acceptable bacteria and fungi can be found in Appendix I, indicating up to date nomenclature and also lists microorganisms that are excluded from the monograph for which further assessment by the Natural and Non-prescription Health Products Directorate of Health Canada is needed prior to marketing.

ANVISA, the national Health Agency in Brazil, for many years had a list of approved probiotics recognized as safe and a standard approved claim "The (stated species of microorganism / probiotic) contributes to the balance of intestinal flora. Its consumption should be associated with a balanced diet and lifestyle habits healthy ". Probiotics which were not part of the list had to prove safety and evidence of a physiological or specific metabolic effect. Recently, ANVISA decided to exclude the list of approved probiotics and defines that the company should propose a functional or health claim and it will be evaluated case by case, based on the definitions and principles set out in Resolution no. 18/1999 ". The existing resolution defines the guidance for analysis and proof of functional and /or health claims on food labels.

<http://portal.anvisa.gov.br/wps/content/Anvisa+Portal/Anvisa/Inicio/Alimentos/Assuntos+de+Interesse/Alimentos+Com+Alegacoes+de+Propriedades+Funcionais+e+ou+de+Saude/Avaliacao+de+seguranca+e+comprovacao+de+eficacia>