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Guidelines for the Evaluation of Probiotics in Food

Report of a Joint FAO/WHO Working Group on

Drafting Guidelines for the Evaluation of Probiotics in Food

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The opinions expressed in this report are those of the participants of the Working Group and do not imply any opinion on the part of FAO and WHO

1. Introduction

The Joint FAO/WHO Expert Consultation on **Evaluation of Health and Nutritional Properties of Probiotics in Food** held in Córdoba, Argentina from 1-4 October, 2001 recognized that there is a need for guidelines to set out a systematic approach for the evaluation of probiotics in food leading to the substantiation of health claims. Consequently, a Working Group was convened by FAO/WHO to generate guidelines and recommend criteria and methodology for the evaluation of probiotics, and to identify and define what data need to be available to accurately substantiate health claims. The aims of the Working Group were to identify and outline the minimum requirements needed for probiotic status. Consequently, guidelines were prepared to meet this objective.

2. Scope

The report of the **Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food** addressed probiotics in food, and excluded reference to the term biotherapeutic agents, and beneficial microorganisms not used in food (http://www.fao.org/es/ESN/Probio/probio.htm). The Working Group adopted the same scope as the Expert Consultation, and specifically excluded genetically modified organisms (GMOs).

3. Guidelines for Probiotics

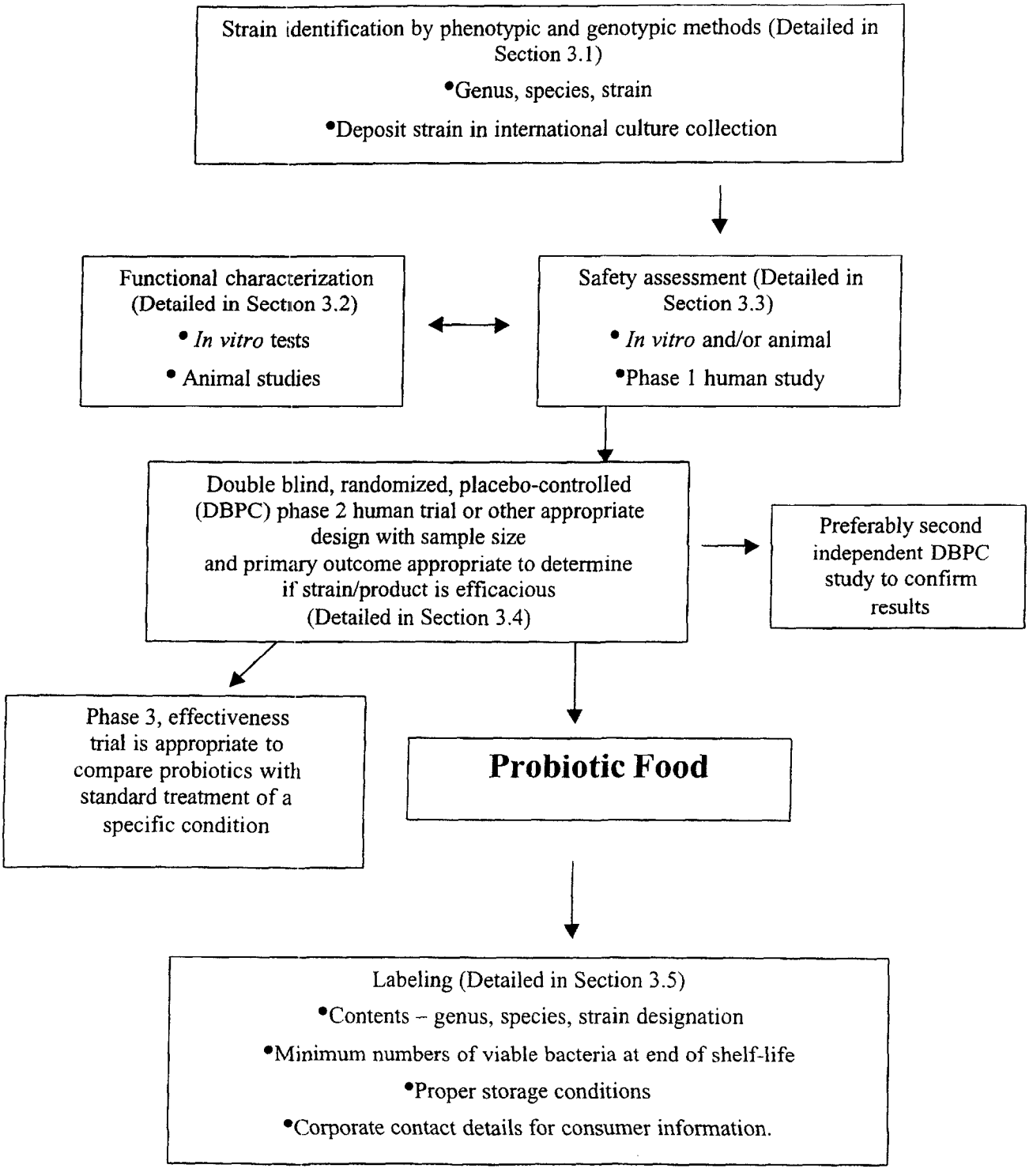
In order to claim that a food has a probiotic effect, the guidelines set forth in this report should be followed. A scheme outlining these guidelines for the evaluation of probiotics for food use is shown in Fig. 1. This was the basis for discussions and details are specified in the following sections of this report.

3.1. Genus/species/strain

It was recognized that it is necessary to know the genus and species of the probiotic strain. The current state of evidence suggests that probiotic effects are strain specific. Strain identity is important to link a strain to a specific health effect as well as to enable accurate surveillance and epidemiological studies. A possible exception is the ability in general of *S. themophilus* and *L. delbrueckii* ssp. *bulgaricus* to enhance lactose digestion in lactose intolerant individuals. In this case, or in other cases where there is suitable scientific substantiation of health benefits that are not strain specific, individual strain identity is not critical.

Speciation of the bacteria must be established using the most current, valid methodology. It is recommended that a combination of phenotypic and genetic tests be used.

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



Nomenclature of the bacteria must conform to the current, scientifically recognized names. Protracted use of older or misleading nomenclature is not acceptable on product labels. The use of incorrect names does not properly identify the probiotic bacterium in the product and forces consumers and regulatory agencies to make assumptions about the identity of the real bacterium being sold. Current nomenclature can be retrieved as follows:

- Approved Lists of Bacterial Names (Int. J. Syst. Bacteriol, 1980,30:225-420) also available in http://www.bacterio.cict.fr/
- Validation Lists, published in the International Journal of Systematic and Evolutionary Microbiology (or International Journal of Systematic Bacteriology, prior to 2000)

DNA-DNA hybridization is the reference method to specify that a strain belongs to a species; however, as it is time consuming and beyond the resources of many laboratories, requiring a large collection of reference strains, the use of DNA sequences encoding 16S rRNA is suggested as a suitable substitute. In this case, it is recommended that this genotypic technique be combined with phenotypic tests for confirmation.

Patterns generated from the fermentation of a range of sugars and final fermentation products obtained from glucose utilization are key phenotypes that should be investigated for identification purposes.

Strain typing has to be performed with a reproducible genetic method or using a unique phenotypic trait. Pulsed Field Gel Electrophoresis (PFGE) is the gold standard. Randomly Amplified Polymorphic DNA (RAPD) can also be used, but is less reproducible. Determination of the presence of extrachromosomal genetic elements, such as plasmids can contribute to strain typing and characterization.

It is recommended that all strains be deposited in an internationally recognized culture collection.

3.2. In vitro tests to screen potential probiotics.

In vitro tests are critical to assess the safety of probiotic microbes (see Section 3.3).

In addition, *in vitro* tests are useful to gain knowledge of strains and the mechanism of the probiotic effect. However, it was noted that the currently available tests are not fully adequate to predict the functionality of probiotic microorganisms in the human body. It was also noted that *in vitro* data available for particular strains are not sufficient for describing them as probiotic. Probiotics for human use will require substantiation of efficacy with human trials. Appropriate target-specific *in vitro* tests that correlate with *in vivo* results are recommended. For example, *in vitro* bile salts resistance was shown to correlate with gastric survival *in vivo* (Conway et al., 1987). A list of the main currently

used *in vitro* tests for the study of probiotic strains is shown in Table 1. All of these tests require validation, however, with *in vivo* performance.

Table 1. Main currently used *in vitro* tests for the study of probiotic strains

Resistance to gastric acidity
Bile acid resistance
Adherence to mucus and/or human epithelial cells and cell lines
Antimicrobial activity against potentially pathogenic bacteria
Ability to reduce pathogen adhesion to surfaces
Bile salt hydrolase activity
Resistance to spermicides (applicable to probiotics for vaginal use)

3.3. Safety considerations: Requirements for proof that a probiotic strain is safe and without contamination in its delivery form

Historically, lactobacilli and bifidobacteria associated with food have been considered to be safe (Adams & Marteau, 1995). Their occurrence as normal commensals of the mammalian flora and their established safe use in a diversity of foods and supplement products worldwide supports this conclusion. However, probiotics may theoretically be responsible for four types of side-effects (Marteau, 2002):

1. Systemic infections
2. Deleterious metabolic activities
3. Excessive immune stimulation in susceptible individuals
4. Gene transfer

Documented correlations between systemic infections and probiotic consumption are few and all occurred in patients with underlying medical conditions. The following is a list (including some microbes used in non-food applications) of infections reported to be associated (although not necessarily proven) with the consumption of commercial products:

- Two cases of *L. rhamnosus* traced to possible probiotic consumption (Rautio et al., 1999; Mackay et al., 1999).
- 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. *Enterococcus* is emerging as an important cause of nosocomial infections and isolates are increasingly vancomycin resistant. The Working Group 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 probiotic strain is not a significant risk with regard to transferable antibiotic resistance or other opportunistic virulence properties.

In recognition of the importance of assuring safety, even among a group of bacteria that is Generally Recognized as Safe (GRAS), the Working Group recommends that probiotic strains be characterized at a minimum with the following tests:

1. Determination of antibiotic resistance patterns
2. Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
3. Assessment of side-effects during human studies
4. Epidemiological surveillance of adverse incidents in consumers (post-market)
5. If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition (SCAN, 2000)
6. If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required

Assessment of lack of infectivity by a probiotic strain in immunocompromized animals would add a measure of confidence in the safety of the probiotic.

3.4. In vivo studies using animals and humans

In some cases, animal models exist to provide substantiation of *in vitro* effects and determination of probiotic mechanism. Where appropriate, the Working Group encourages use of these prior to human trials.

The principal outcome of efficacy studies on probiotics should be proven benefits in human trials, such as statistically and biologically significant improvement in condition, symptoms, signs, well-being or quality of life; reduced risk of disease or longer time to next occurrence; or faster recovery from illness. Each should have a proven correlation with the probiotic tested.

Probiotics have been tested for an impact on a variety of clinical conditions (see Expert Consultation Report, Section 5.3). Standard methods for clinical evaluations are comprized of Phase 1 (safety), Phase 2 (efficacy), Phase 3 (effectiveness) and Phase 4 (surveillance). Phase 1 studies focused on safety are discussed in Section 3.3 above. Phase 2 studies, generally in the form of randomized, double blind, placebo-controlled (DBPC) design, measure efficacy compared with placebo. In addition, phase 2 studies measure adverse effects. A general recommendation for the testing of probiotic foods is that the placebo would be comprized of the food carrier devoid of the test probiotic. Sample size needs to be calculated for specific endpoints. Statistically significant differences must apply to biologically relevant outcomes.

Probiotics delivered in food generally are not tested in Phase 3 studies, which are concerned with comparison with a standard therapy. When a claim is made for a probiotic altering a disease state, the claim should be made based on sound scientific evidence in human subjects.

In Phase 2 and 3 studies, the Working Group recognizes the value of validated quality of life assessment tools.

It is recommended that human trials be repeated by more than one Center for confirmation of results.

No adverse effects related to probiotic administration should be experienced when food is considered. Adverse effects should be monitored and incidents reported.

The Working Group recommends that information accumulated to show that a strain(s) is a probiotic, including clinical trial evidence be published in peer-reviewed scientific or medical journals. Furthermore, publication of negative results is encouraged as these contribute to the totality of the evidence to support probiotic efficacy.

Further information on the generation and use of clinical information to substantiate health effects can be found at www.ftc.gov/bcp/conline/pubs/buspubs/dietsupp.htm#IIb

3.5. Health claims and labeling

Currently in most countries, only general health claims are allowed on foods containing probiotics. The Working Group recommends that specific health claims on foods be allowed relating to the use of probiotics, where sufficient scientific evidence is available, as per the guidelines set forth in this report. Such specific health claims should be permitted on the label and promotional material. For example, a specific claim that states that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that states 'improves gut health'. This would better comply with Codex General Guidelines on Claims (CAC/GL 1-1979 (Rev. 1-1991) to avoid misleading information.

It is recommended that it be the responsibility of the product manufacturer that an independent third party review by scientific experts in the field be conducted to establish that health claims are truthful and not misleading.

The Working Group recommends that the following information be described on the label:

- Genus, species and strain designation. Strain designation should not mislead consumers about the functionality of the strain
- Minimum viable numbers of each probiotic strain at the end of the shelf-life
- The suggested serving size must deliver the effective dose of probiotics related to the health claim
- Health claim(s)
- Proper storage conditions
- Corporate contact details for consumer information

4. Recommendations

1. Adoption of the definition of probiotics as 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'.
2. Use and adoption of the guidelines in this report should be a prerequisite for calling a bacterial strain 'probiotic'.
3. Regulatory framework to allow specific health claims on probiotic food labels, in cases where scientific evidence exists, as per the guidelines set forth in this report.
4. Promotion of these guidelines at an international level.
5. Good manufacturing practices (GMP) must be applied in the manufacture of probiotic foods with quality assurance, and shelf-life conditions established.
6. Further development of methods (*in vitro* and *in vivo*) to evaluate the functionality and safety of probiotics.

5. List of Abbreviations

CAC/GL: Codex Alimentarius Commission/General Guidelines on Claims

DBPC: Double blind, randomized, placebo-controlled

DNA: Deoxyribonucleic Acid

FAO: Food and Agriculture Organization of the United Nations

GMO: Genetically Modified Organism

GMP: Good manufacturing practices

GRAS: Generally Recognized as Safe

PFGE: Pulsed Field Gel Electrophoresis

RNA : Ribonucleic Acid

RAPD: Randomly Amplified Polymorphic DNA

SCAN: EU Scientific Committee on Animal Nutrition

WHO: World Health Organization

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Annex 1

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