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Artículo especial

Consensus statements from the Workshop "Probiotics and Health: Scientific Evidence"

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Abstract

This report shows the level of scientific consensus on definition, characteristics and health benefits of probiotics. The content of the report has derived from the scientific meeting: Workshop on Probiotics and Health. Scientific evidence, that congregated several Spanish experts, including gastroenterologists, microbiologists, nutritionists, immunologists and food technologists, among others, who have agreed with the statements shown in this document. Each statement has been sustained with the most relevant scientific aspects that were discussed during the Workshop and the following evaluation of the report by all experts who approved and signed it.

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DECLARACIONES CONSENSUADAS EN EL WORKSHOP "PROBIÓTICOS Y SALUD: EVIDENCIA CIENTÍFICA˝

Resumen

En este documento se muestra una base de consenso en torno a la definición, características y propiedades beneficiosas de los probióticos. El contenido fue generado a partir de la reunión científica Workshop Probióticos y Salud. Evidencia Científica, que agrupó a una variedad de expertos españoles gastroenterólogos, microbiólogos, nutricionistas, inmunólogos y tecnólogos de alimentos, entre otros, que se han adherido en su mayoría a las sentencias que constituyen este documento. Para cada sentencia se establecen las aspectos científicos más relevantes que la respaldan y que son consecuencia del acuerdo al que se ha llegado tras el debate surgido en la reunión y la evaluación posterior del contenido por todos los expertos que han firmado este documento.

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Palabras clave: *Probiótico. Evidencia científica. Salud gastrointestinal. Inmunomodulación.*

1. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host

This definition was proposed by a Joint FAO/WHO working group of experts,[1] and has been widely accepted in the scientific literature, being the most referenced definition since it was first published. In general, aspects such as the viability of microorganisms, oral administration and demonstrated beneficial effects on health after consumption are permanent criteria in most definitions that have been proposed for probiotics. Although not explicitly reflected, the definition also implies that probiotics should survive passing through the gastrointestinal tract and preserve their beneficial capacity when they reach the sites where they interact with the host, which explains their mechanisms of action. The definition also implies the viability of probiotics throughout the shelf life of the product in which they are administered. Products should contain the quantity of microorganisms needed to provide the benefit.

2. Those substances that are constituents of microorganisms or are produced by them should not be considered probiotics even if they would produce a beneficial effect on health

Some mechanisms of action relative to beneficial effects of probiotics include, among others, the

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release of microbial enzymes in the intestine (e.g. β-galactosidase, which hydrolyses the lactose present in foods), the secretion of proteins and the extracellular proteins or other macromolecules associated to microbial envelopes that interact with pattern-recognising receptors in host cells[2] or the production of metabolites or peptides with antimicrobial activity (e.g. bacteriocins). Although they are related to the mechanisms of action by which probiotic microorganisms exert their beneficial effect on the host, these substances should not be considered to be probiotics themselves because they are not live or viable organisms. Likewise, part of the resident or autochthonous intestinal microbiota can enable certain metabolic activities that human intestinal cells cannot, such as the degradation of non-digestible components in the diet, methanogenesis, gluconeogenesis, and detoxification of xenobiotics or the biosynthesis of essential amino acids, vitamins and isoprenoids. However, such activities do not necessarily confer to these microorganisms the qualification of probiotics.

3. For a microorganism to qualify as a probiotic, it must be scientifically demonstrated that it produces beneficial effects on the health of the host

The ability to produce a beneficial effect on health is an essential property that characterises a probiotic. In the 21st century, it is clear that an effect on health can and must be verified by scientific methods which guarantee the efficacy and safety of the agent which produces such effect. Therefore, health claims which are not based on scientific evidence[3] are not acceptable. Thus, there must be scientific evidence showing that the consumption of a certain microorganism produces a specific health effect in order to support the probiotic status.

4. Beneficial effects on health should be demonstrated through studies carried out on the human population with suitable scientific methodology

The usual methods to provide the scientific evidence of a certain effect were reviewed both by the group of experts from WHO and FAO,[1] as well as by the working groups from PASSCLAIM;[3] these two publications therefore offer an extensive view on the matter. There is unanimous consensus on the concept that there is no definitive proof of a given health effect if its efficacy has not been confirmed in individuals of the same species for which its use is intended. Therefore, the probiotics which are intended for human health must have proven efficacy in studies with human subjects.

5. Laboratory or animal model studies are a necessary requirement before performing studies in the human population, and they provide information regarding mechanisms of action; however, alone they should not be considered as sufficient proof of efficacy in human health

The unavoidable need to demonstrate efficacy and safety in studies carried out in human subjects should not lead to the conclusion that laboratory or animal model studies are superfluous or even useless. In the case of probiotics, it is always necessary to start with an adequate characterisation of the microorganism that is intended to be selected for its use in humans. The adequate characterisation of the microorganism, according to the recommendations of the WHO/FAO group,[1] must include its identification, including genus, species and strain using phenotypic methods (biological properties expressed *in vitro*) and genotypic methods (sequencing of its genes, especially of the 16SrRNA gene fragments, and a description of methods for its identification in biological media by specific techniques including fingerprinting techniques such as PFGE or similar techniques) and depositing the strain in internationally recognised collections. Adequate characterisation and safety studies in animal models are necessary before use in the human population. Moreover, laboratory and animal model studies are ideal for understanding and illustrating the mechanism of action of a given probiotic on human health.

6. Healthy effects demonstrated for a specific microbial strain cannot be extrapolated or attributable to other strains of the same species

A microbial strain is defined by the different properties and characteristics that differentiate it from other microorganisms of its species, including phenotypic properties (morphological, physiological, biochemical, ecological, etc.), structural properties (cytoplasmic membrane, cell wall, capsule, etc.) and genetic properties (DNA, RNA). The strains that result from new microbial isolates should be evaluated to demonstrate whether a beneficial effect is present, regardless of whether they correspond to a species in which a probiotic strain has been reported. The development of molecular biology methodologies has led to the identification of different effector cell and molecule structures that are strain-dependent and which are related to the specific interactions between probiotic microorganisms and host cells.[4] Some strain-dependent characteristics that have been described are, among others, the production of bacterial adhesins and exopolysaccharides, related to the probiotics which remain in the intestine, immunomodulating activity and the production of bacteriocins. Furthermore, the strain-specific characteristics of probiotics makes it necessary to develop DNA fingerprinting techniques in order to

assess the authenticity of the microorganism claimed to be a probiotic, as well as its viability in the products in which it is administered.

7. A microbial strain properly qualified as probiotic due to its demonstrated efficacy for a specific indication (e.g. diarrhoea prevention) is not necessarily valid for other indications (e.g. allergy prevention)

Strains included in the probiotic category, depending on their characteristics, must have been proven effective for a specific condition. This is the case of *Lactobacillus rhamnosus GG,* the usefulness of which has been demonstrated in several studies on paediatric gastroenteritis and diarrhoea associated with treatment with antibiotics.[5-7] Nevertheless, there are no recommendations for its use in other situations such as genitourinary infection prevention[8]. Randomised, placebo-controlled studies for intervention with *L. rhamnosus GG*, carried out to compare the preventative capacity of developing eczema and atopic sensitisation in at-risk children have given rise to contradictory results for eczema prevention in general and, in particular, negative results for the prevention of eczema with atopic sensitisation.[9-12]

8. The effectiveness of some probiotics strains is widely documented for specific gastrointestinal health conditions (e.g. some types of diarrhoea, constipation, irritable bowel and intestinal inflammation)

One main area in which probiotics have been applied is in the prevention and treatment of gastrointestinal disorders. There is extensive scientific evidence documenting the efficacy of certain probiotic strains in acute processes of the digestive tract. Some of these indications are already common in medical practice. In contrast, the use of probiotics or prebiotics in chronic gastrointestinal processes is still much more limited. Recently, the World Gastroenterology Organisation (WGO) published a practical guide on the use of probiotics and prebiotics in gastroenterology.[13] The WGO guide was drafted by an international group of experts and translated into several languages; it is available on the organisation's website in English, French, Spanish, Portuguese, Russian and Mandarin Chinese. It is worth noting that the clinical guide includes a table of specific conditions that can be treated with certain probiotics, thereby avoiding generalised recommendations which are not based on scientific findings.

9. There are probiotic strains with demonstrated efficacy for specific indications of the immune system (e.g. prevention of infections)

Today, it is generally accepted and has been demonstrated by both *in vitro* and *in vivo* experiments that probiotic strains interact with the immune system of the host.[14] For example, the formulation of multiple species contained in the probiotic preparation VSL#3 has shown a beneficial anti-inflammatory effect in mice by way of local stimulation of epithelial innate immune responses (increase in the synthesis of epithelial TNF-α and reestablishment of intestinal permeability).[15]

Effects on the immune system that have been repeatedly observed in well-conducted intervention studies in human beings include:

- **Modulation of phagocytic activaty**. *Lactobacillus johnsonii* La1 may limit low-grade chronic inflammation due to overgrowth of intestinal bacteria in the elderly, decreasing phagocytosis.[16] *L. rhamnosus* HN001 has demonstrated its ability to modulate innate cell immunity, increasing the phagocytic capacity of polymorphonuclear cells (PMN) and monocytes, as well as an increase in the phagocytic activity of natural killer cells (NK) in adults and the elderly.[17,18] The same has been demonstrated for *Bifidobacterium lactis HN019*, although the two studies that have documented this were carried out in a small number of subjects.[19,20]

- **Coadjuvant effect in vaccination**. In studies on the expression of activation markers and cytokine production in *in vitro* cultures of dendritic cells (derived from monocytes) and cocultivated T-cells with different species of lactobacillus (*L. johnsonnii*, *L. reuteri*, *L. gasseri*), it has been demonstrated that dendritic cells activated as such secrete interleukin (IL)-12 and IL-18 and polarise the response of lymphocytes toward a Th1-type response by inducing the secretion of interferon (IFN)-γ but not that of IL-4 or IL-13.[20] The same has been observed with *Lactobacillus fermentum CECT5716* as its administration together with anti-flu vaccination improves Th1 response and the production of antibodies.[22] Likewise, an adjuvant effect in vaccination against the seasonal flu has also been shown in milk fermented with *Lactobacillus casei DN 014 001* and live cultures in yoghurt.[23]

- **Mitigation of common winter infections**. Although various studies carried out with probiotics have highlighted this positive effect on health, it cannot be attributed to one single species or strain because the evidence available comes either from studies that use combinations of bacteria species,[24] or studies that use one single strain, such as *L. johnsonii* La1[25]; it would be advisable to reproduce the evidence by subsequent independent studies.

10. Scientific evidence observed for one population group cannot be extrapolated to other populations of different age (children and the elderly) or physiological state (e.g. pregnancy and breastfeeding)

Several studies have attempted to demonstrate the efficacy and safety of probiotic in different population groups and physiological states. The studies which have demonstrated the scientific evidence of probiotics can only be attributed to the strain(s) analysed in each population group studied; they cannot be generalised for all populations and physiological states.[26] The anatomical and physiological differences (not only in the intestinal microbiota) between children, adults and the elderly in the healthy population are significant, which is why every study must normally attempt to demonstrate the efficacy of probiotic bacteria in samples representative of specific population groups. The same can be said for special physiological states such as pregnancy and breastfeeding. In any case, the meta-analyses which have included different population groups have found different, non-extrapolative effects. In this respect, a meta-analysis carried out by Sazawal et al.[27] found that the use of probiotics to prevent acute diarrhoea is more effective in children than in adults. Therefore, not all probiotics (or the combination thereof) act equally from one given population group to another, or in one physiological state or another, making it therefore necessary to demonstrate their effects in studies that are properly designed for each situation.

Experts who have agreed to join the consensus:

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