Ministero della Salute

DIREZIONE GENERALE PER L'IGIENE E LA SICUREZZA DEGLI ALIMENTI E LA NUTRIZIONE - UFFICIO 4

GUIDELINES ON PROBIOTICS AND PREBIOTICS
Revised in March 2018

INTRODUCTION

In Italy, lactic bacteria started being used as food supplements about 35 years ago, when, in accordance with the legislation in force at the time, these products were classified in the category of dietetic products and needed to be authorized before marketing.
The first products that were authorized contained *Saccharomyces cerevisiae* or lactic ferments such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus,* combined with nutrients, mostly B vitamins, which gave them the "nutritional" value that was at the time deemed required to classify them as dietetic products.
The above mentioned category of products was identified as "*integratori dietetici biologico-vitaminici*" (biological-vitamin food supplements) for which a "disciplinare ministeriale" (thus it was called at the time a specific guideline on the requirements that had to possess a certain categories of dietary products) laid down the minimum intake of live cells with the recommended daily consumption quantity in order to be allowed to claim any intestinal flora balance restoring effect in the label.
In this perspective, the combination with vitamins had its reason why in the usefulness of concomitant supplementing the expected deficit in their bacterial synthesis subsequent to the imbalance of the intestinal ecosystem.
Afterwards, the term "*biologico*", [which in Italian means both "biological" and "organic", *translator's note*], was progressively abandoned because of its being in conflict with the term "*biologico*" meaning organic when used for "*agricoltura biologica*" (organic farming) products and was replaced with the term "*probiotic*o" (probiotic).
Since 2002, with the entry into force of Directive 2002/46/EC on food supplements, the scope of which was extended also to "*concentrated sources*" of substances having "*physiological effects*", products based only on "*probiotics*" with no combined nutritional components have been admitted as food supplements.
In Italy, the "*physiological*" effect, aimed at favouring the intestinal flora balance, has always been considered useful for health and bound to the ability of a probiotic to colonize in the intestines, thanks to the intake of a sufficient number of live cells with the recommended daily consumption quantity.

When assessing the claims to be authorized pursuant to Regulation (EC) 1924/2006, the EFSA opinion reported that "*Increasing the number of any groups of bacteria" as well as "enhance levels of beneficial microflora"* are not in itself "*beneficial to human health, and that to support a balanced/beneficially affect intestinal microflora in the context of decreasing potentially pathogenic*

intestinal microorganisms might be beneficial to human health"

The EFSA Journal 2009; 7(9) 1232 quotes: "*Increasing the number of any groups of bacteria in not in itself considered as beneficial. The Panel considers that no evidence has been provided that ..... enhance levels of beneficial microflora are beneficial to human health. The Panel considers that support a balanced/beneficially affect intestinal microflora in the context of decreasing potentially pathogenic intestinal microorganisms might be beneficial to human health".*

According to the aforementioned approach, the documentation of probiotic colonization in the intestines, as evidence of its useful action for restoring the intestinal flora, is not, in itself, sufficient evidence to claim any beneficial effect on health pursuant to Article 2.2.5 of Regulation (EC) 1924/2006.
Reasserting the validity and proportionality of the Italian approach to probiotics to recognize their "effectiveness" in physiological terms, it is however pointed out that the indication of a probiotic for promote the balance of intestinal flora", in accordance with the conditions provided for by these guidelines, is not a health claim that can be authorized pursuant to Article 13.5 of Regulation (EC) 1924/2006.
The same consideration may be made for "*prebiotics*", based on their composition and the set of scientific evidence supporting their indication for physiological effects on the bacterial flora balance.
Given all the above, it is understood that, since they are presumably able to foster the bacterial flora balance, the products that are compliant with the present guidelines in terms of their content of probiotics or prebiotics can claim such physiological effect in the label as "**probiotic**" and "**prebiotic**".

1. PROBIOTICS

Indications for use in food products and food supplements of probiotic micro-organisms (bacterial and/or yeasts), which have long been used to promote the balance of intestinal microflora

1.1. Characteristics of the micro-organisms that may be used in food products and food supplements

The micro-organisms that may be used in food products and food supplements shall comply with the following requirements:

a) Having long been used to supplement human intestinal microflora (microbiota);

b) Being considered safe for use in humans.
To this end, a useful benchmark consists in the criteria set by EFSA on the "Qualified presumption of safety" ("QPS") status. In any case, the micro-organisms used to manufacture food products shall not be carriers of acquired and/or transmissible antibiotic-resistance;

c) Being active in the intestines in such a quantity as to be able to multiply there (see section 1.3 "Quantity of micro-organisms").

1.2. Characterization *

The assessment of the taxonomic position ensures that the micro-organism used is safe because it allows recognition of a species with a long record of safe consumption.

1.2.a Bacteria characterization

Species identification

Analyzing the sequence of validated taxonomic markers, including, where necessary, at least two of them (for instance the complete genetic sequence of the encoding gene for the16S rRNA) or the complete genomic sequence or other internationally accepted molecular methods

Strain identification
Through macro-restriction of the chromosomal DNA followed by pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), analysis of Random Amplified Polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), Whole Genome Mapping (WGM) or the analysis of imaging genomic mapping, analysis of the validated fully assembled genome sequence, or other internationally accepted genetic typing molecular methods.

The bacterium is considered sufficiently characterised only when both criteria are met.

1.2.b Yeast characterization

Species identification
Through the analysis of the sequence of DNA-based taxonomic markers (for example domains D1 and D2 of 26S rRNA or of spacers [ITS] between the regions of the subunits of the rRNA gene, including gene 5.8S rRNA), analysis of Restriction Fragment Length Polymorphism (RFLP) (for instance RFLP of 5.8S r.DNA ITS or RFLP of mitochondrial DNA), analysis of the validated fully assembled genome sequence, or other internationally accepted genetic typing molecular methods.

Strain identification
Through the analysis of PFGE chromosome length polymorphism, RAPDs, analysis of microsatellite DNA polymorphism, analysis of the validated fully assembled genome sequence, or other internationally accepted genetic typing molecular methods

Only when both criteria are met, the yeast is considered sufficiently characterized

The species shall be named using the taxonomic nomenclature recognized by the International Union of Microbiological Societies (IUMS).
Moreover, it is recommended to deposit strain in international Collections that qualify as International Depositary Authority (IDA).

*
EFSA 2016.4367. "General scientific guidance for stakeholders on health claim applications" Annex B
EFSA 2009. Scientific opinion on the substantiation of health claims related to non-characterised microorganisms pursuant to Article 13 of Regulation (EC) No 1924/2006 on request from the European Commission. EFSA Journal 2009;7(9):1247, 64 pp. doi:10.2903/j.efsa.2009.1247 Available at http://www.efsa.europa.eu/en/efsajournal/pub/1247
EFSA 2010c. Scientific Opinion on the substantiation of health claims related to non-characterised bacteria and yeasts pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Journal 2010;8(2):1470, 44 pp. doi:10.2903/j.efsa.2010.1470 Available at http://www.efsa.europa.eu/en/efsajournal/pub/1470

1.3 Quantity of micro-organisms
Based on the available scientific evidence, the minimum quantity that is sufficient to obtain temporary colonization of intestines by a microbial strain is at least 10^9 live cells per day. Therefore, the recommended product serving for daily consumption shall contain a quantity of 10^9 live cells of at least one of the strains. Use of lower quantities may be allowed only if adequate scientific studies support the ability of the strain used to colonize the intestines.

The quantity of live cells in the product shall be stated on the label for each strain and shall be guarantees, in accordance with the suggested storage methods, until the end of the product shelf-life, with uncertainty of 0.5 log. It is pointed out that the most suitable analysis methods to quantify

live micro-organisms may vary according to each species.

1.4 Safety of probiotics

The use of any new microbial strain, albeit belonging to a species already in use, requires a new assessment of safety and effectiveness, in accordance with its ability to colonize.

As regards safety assessment, it is reasserted that taxonomic identification in terms of species and strain is required, using the aforementioned techniques, as is the assessment of antibiotic-resistance profile (antibacterial or antimycotic, as the case may be). The antibiotic-resistance profile shall be determined for each single microbial strain used, in order to exclude acquired ones and even potentially transmissible ones. As an exception, assessing the safety of a strain that belongs to sufficiently characterized species is not deemed necessary, as defined by the EFSA documents on the QPS status of some bacterial groups. Also in this case, however, the antibiotic-resistance profile is to be assessed.

1.5 Indications for use

It promotes the intestinal flora balance (favorisce l'equilibrio della flora intestinale)

2. PREBIOTICS

Indications for use in food products and food supplements of prebiotics that have long been used for the balance of the intestinal flora

2.1 Definitions

These indications make reference to the FAO document "FAO Technical Meeting on Prebiotics" (Rome, September 2007), where the term "prebiotic" is defined as follows:

"*A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microflora*".

The substances used as prebiotics shall comply with the following requirements:

- Being safe for human consumption based on a long-standing use that prevents it from being classified as novel food pursuant to Regulation (EU) 2015/2283;
- Being contained in recommended daily consumption amounts in quantities that are plausibly fit to generate a "prebiotic" effect in accordance with the available scientific evidence.

Some examples of constituents that can be used as prebiotics are inulin, fructooligosaccharides (FOS) and galacto-oligosaccharides (GOS)

2.2 Indication for use

It promotes the intestinal flora balance (favorisce l'equilibrio della flora intestinale)