Introduction

With the growing interest in self-care and integrative medicine coupled with our health-embracing increasing population, recognition of the link between diet and health has never been stronger. As a result, the market for functional foods, or foods that promote health beyond providing basic nutrition, is flourishing. Within the functional foods movement is the small but rapidly expanding arena of probiotics [1]. That’s why in the last years an increased interest is observed not only within the consumers and the food industry, but also within the scientific community. The scientists aim to understand the interactions between the gut and intestinal microbiota and between resident and transient microbiota that define a new arena in physiology, which would shed light on the “cross-talk” between humans and microbes [2].

The increased interest and usage of probiotic products prompted us to summarize the information about their health benefits, classification, quality assurance criteria and methods of analysis and quality control.

Definition of probiotic and related terms

**Probiotic**

The term probiotic, meaning “for life,” is derived from the Greek language. It was first used by Lilly and Stillwell [3] in 1965 to describe “substances secreted by one microorganism which stimulates the growth of another” and thus was contrasted with the term antibiotic. It may be because of this positive and general claim of definition that the term probiotic was subsequently applied to other subjects and gained a more general meaning. In 1971 Speriti [4] applied the term to tissue extracts that stimulate microbial growth. Parker [5] was the first to use the term probiotic in the sense that it is used today. He defined probiotics as “organisms and substances which contribute to intestinal microbial balance.” In our days we accepted the definition of World Health Organization (WHO). According to WHO, probiotics are “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” [6].

**Prebiotic**

The term prebiotic was introduced by Gibson and Roberfroid [7] who exchanged “pro” for “pre,” which means “before” or “for.” They defined prebiotics as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon”. Modification by prebiotics of the composition of the colonic microflora leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, lactobacilli and bifidobacteria [7]. The prebiotics most commonly used are oligosaccharides whose degree
of polymerization varies between 2 and 20 monomers e.g., fructo-oligosaccharides (FOSs), inulin, galacto-oligosaccharides (GOSs) and xylo-oligosaccharides (XOSs) [8].

**Synbiotic**

The term synbiotic is used when a product contains both probiotics and prebiotics. Because the word alludes to synergism, this term should be reserved for products in which the prebiotic compound selectively favors the probiotic compound. The expectation is that the prebiotics will enhance the survival and growth of the probiotics [9, 10].

**Taxonomy of probiotics**

The taxonomy of probiotic lactic acid bacteria was formed according to morphological, biochemical and physiological characteristics with molecular-based phenotypic and genomic techniques. The most studied are genera *Lactobacillus*, *Bifidobacterium* and *Enterococcus* (Table 1). In the genus *Lactobacillus* the following species of importance as probiotics were investigated: *L. acidophilus* group, *L. casei* group and *L. reuteri/L. fermentum* group. *Bifidobacterium* spp. (*B. animalis*) strains have been reported to be used for production of fermented dairy and recently of probiotic products. From the genus *Enterococcus*, probiotic *Ec. faecium* strains were investigated with regard to the vanA-mediated resistance against glycopeptides [11-13].

**Health benefits of probiotics**

Microbiota balance is the oldest proposed probiotic benefit. Metchnikoff defined it as ‘seeding’ of the intestinal tract with harmless lactic acid bacteria (LAB) that suppress the growth of harmful proteolytic bacteria. Nowadays such a benefit is usually interpreted as an increase in lactobacilli and/or bifidobacteria and a decrease in potentially pathogenic bacteria [15]. One of the best studied examples of how microbiota dysbiosis affects health is seen in Crohn’s disease (CD) [16].

Since the early studies of mucosal immunity in the 1970s, a lot of progress has been made in understanding the mode of interaction between the gut microbiota and the immune system. The ability of exogenous probiotics to improve clinical outcomes through modulation of the immune response has been demonstrated in subjects with chronic and acute diseases [17-21]. Immune stimulatory effects have been established in generally healthy population as well [22-28]. A few studies have shown a link between activated immune markers and improved resistance to infections [15].

The gastro-intestinal discomfort, including diarrhea in children and adults, antibiotic-associated diarrhea, constipation and irritable bowel syndrome, can be influenced using probiotic products [29-39]. Some studies report successful treatment of colicky symptoms, frequent in newborn infants, was achieved using probiotic [40].

*Candida* species is the most common cause of vaginal infection, with frequent recurrence and chronic infection, so probiotics may be useful for treatment [41-44]. Studies conducted by Hilton et al. showed a significant reduction in vaginal colonization with *Candida* species after the oral administration and a 7-day course of vaginal suppositories [45, 46].

Other examples of health benefits attributed to probiotics range are prevention of tumours in mice [47, 48], treatment of food allergy including atopic eczema [49], delaying the effects of aging in humans [50] and modulating blood lipid levels [51].

To exert health benefits probiotics must be able to survive transition through the low pH in the stomach and colonize the colon apart from the high concentrations of bile acids [52].

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**Table 1. Microorganisms used as probiotic agents [14]**

<table>
<thead>
<tr>
<th>Lactobacillus species</th>
<th>Bifidobacterium species</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. acidophilus</em></td>
<td><em>B. bifidum</em></td>
<td><em>Bacillus cereus</em></td>
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<tr>
<td><em>L. casei</em> (rhamnosus)</td>
<td><em>B. longum</em></td>
<td><em>Escherichia coli</em></td>
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<tr>
<td><em>L. reuteri</em></td>
<td><em>B. breve</em></td>
<td><em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td><em>L. bulgaricus</em></td>
<td><em>B. infantis</em></td>
<td><em>Enterococcus faecalis</em></td>
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<tr>
<td><em>L. plantarum</em></td>
<td><em>B. lactis</em></td>
<td><em>Streptococcus thermophilus</em></td>
</tr>
<tr>
<td><em>L. johnsonii</em></td>
<td><em>B. adolescentis</em></td>
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<td><em>L. lactis</em></td>
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Safety of probiotics

The application of probiotic microorganisms in foodstuffs requires a thorough safety assessment [53]. The safety assessment is available in some guidelines [54-57]. Data requirements to assess the safety of probiotics can vary depending on the bacterial species of interest, the intended application/use and/or the target populations. Parameters such as taxonomy and identification, phenotypic characterization, history of food use, and human exposure, and so on, are generally considered important. If no history of safe use can be demonstrated, extensive preclinical studies, including standard 90-day toxicity studies as defined in OECD Testing Guideline 408, should also be considered. Clinical studies should include parameters to demonstrate safety in use or tolerability in the target population(s) [53].

Criteria for functional quality assurance and analytical approaches for characterization and validation of probiotic products

The criteria currently used to select probiotics define the optimal quality control of probiotic strains in industrial practice. Important quality-control properties that must be constantly controlled and optimized are the following: adhesive properties; bile and acid stability; viability and survival throughout the manufacturing process; effects on carbohydrate, protein, and fat utilization; and, especially, colonization properties and immunogenicity. Most of these properties are related to the physiologic properties of the strain, but long-term industrial processing and storage conditions may influence probiotic properties. Thus, in addition to technologic properties, functional properties should be considered in quality-control measures [58].

The methods available for detection, enumeration and identification of microorganisms could be also applied to probiotic microorganisms.

Molecular methods

Identification of probiotic species, including their differentiation in different strains by culture-dependent or culture-independent techniques, the study of microbial community composition, and assessment of its different populations and interactions in food products are some of the main achievements of molecular techniques. With the use of molecular methods, the ability to detect and to identify food microbes, including probiotic bacteria, has made tremendous advances in recent years, especially after the introduction of PCR (polymerase chain reaction) in the 1980s. Several detection techniques have been developed based on PCR, namely denaturing gradient gel electrophoresis (DGGE), real-time PCR (qPCR), terminal restriction fragment length polymorphism (T-RFLP), random amplified polymorphic DNA (RAPD) [8, 59].

In Figure 1 is presented the approach of the molecular analysis.

To rapidly detect multiple microorganisms in a single reaction, simultaneous amplification of more than one locus is required; a methodology referred to as multiplex PCR (MPCR) in which several specific primer sets are combined into a single PCR assay. Hence, MPCR is undoubtedly useful to rapidly identify several isolates and, with respect to DGGE, it enables the selection of various species and represents the fastest culture-independent approach for strain-specific detection in complex matrices. At a certain level, MPCR might be considered a quantitative (or better yet, a semi-quantitative) technique, since, once it has been established, the detection limit can be retrieved and used as the minimal microbial concentration detectable [60].

Non-molecular methods

The non-molecular approach to detect, to enumerate and to identify probiotic strains includes those methods considered conventional, based on the growth of the microorganism in culture media, and those not requiring this step (Fig. 1). The basis of the conventional methods relies on standard procedures encompassing isolation, counting and identification of the probiotic strains at the genus and species levels based on cell ability to reproduce and to form colonies on selective/differential agar media plates [61].

Alternative methodologies have been applied to quantify probiotic bacteria in a more accurate way able to reduce the underestimation of viable bacteria obtained by plate-count methods. Fluorescence methods have been applied in bacterial viability studies [62]; Fluorescence methods are able to detect and to differentiate between viable, injured, stressed and dead bacterial cells through fluorescent dyes, which, in turn, can be detected by fluorescence microscopy, fluorometry, flow cytometry, or fluorescence in situ hybridization (FISH).

An alternative method is Fourier transform infrared (FT-IR) spectroscopy that has been applied for detection, discrimination, identification and classification of lactic acid bacteria (LAB) and probiotics and also provides information about cell metabolism from cultures and foods. FT-IR is able to discriminate viable, injured
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and dead bacteria and is used in the analysis of structural components of bacteria. FT-IR is a relatively fast, simple, sensitive technique, requiring very little sample, and the biological cell remains intact during analysis. It is possible to make qualitative and quantitative analysis and the sample can be in the form of liquid, gas, powder, solid, or film. If the sample is complex, then it can produce overlapping spectra, and so previous separation or purification steps may be needed or may require standardization, rigorous data collection, and expertise in the chemometric analyses of spectra [63, 64]. In Figure 2 is presented the approach of the non molecular analysis.

**Applications & Dosage Forms**

As discussed above the most common species of probiotics used in foods are **Lactobacilli** and **Bifidobacteria**. The same **Lactobacilli** and **Bifidobacteria** are used in both foods and food supplements. In addition some **Enterococcus** species, **Bacillus** species, **Escherichia coli**, **Saccharomyces boulardii** (a yeast), and other species are used in food supplements [65].

A wider variety of probiotic strains, either singly or in combination, is used in supplements rather than in foods. Several product formulations exist, like: hard gelatin or vegetable capsules, tablets with or without enterocoating (e.g., those that dissolve in neutral conditions), chewable tablets, and sachets. Supplement formulations may also contain other active components, including vitamins and prebiotics. Probiotics are also found as sold in oil suspensions, which makes them easy to administer to infants. Furthermore, probiotics are combined with oral rehydration salts for the treatment of acute diarrhea, whereas some of these products are registered as drugs [65].

**Fig. 1. Molecular approach for identification of probiotic species.**
Probiotics are worldwide available, as supplements consisting of freeze-dried bacteria, secondly in fermented foods such as yoghurts, and thirdly in products aimed at enhancing specific aspects of ‘health’, such as bowel cleansers. In some countries probiotics are also sold as remedies for specific medical conditions such as diarrhea [66].

Not surprisingly, in some late experiments of probiotic products have been found that about 70 to 80 percent of the samples tested do not measure up to their label claims. In these experiments is also established, that about half of the tested samples did not have even 10 percent of the claimed number of live microorganisms as listed on their labels. In some products tested have even been determined a presence of undesirable microorganisms not listed on the label [67]. Keeping this in mind, in order to obtain well produced product it is essential to follow the following steps, when producing: eliminating oxygen from and including nitrogen in probiotic supplement bottles to enhance the stability of probiotics; probiotic supplements should be refrigerated to maintain their potency and viability; any new bacterial culture that has no history of prior safe use in humans should be subject to toxicological studies prior to incorporation in any probiotic supplement and also selecting acid resistant strains of is the key to the success of the probiotic supplement [67].

In order to assure the qualities of the probiotic product, it is important to know that the supplement is tested for viable microorganisms at the time of manufacturing and at the expiration date. This quality control procedure is important to the manufacturer as well as the consumer. The viable cells are guaranteed as CFU (colony forming units) per gram at the time of probiotic supplement packaging. If the supplement does not list viable cells, or does not list the amount in CFU form, it may not be a quality supplement. Consumption of probiotic supplements with two to five billion CFU per day is necessary to have any chance of offering significant beneficial effects [67].

In conclusion in order for a consumer to be sure of the quality and safety of the probiotic product, the following tips should be considered:
- All probiotic supplements lose their potency when they exposed to oxygen, moisture, and...
heat. The probiotic strain must also be proven to survive stomach acids in live human subjects. The bacteria strain should be able to adhere to the intestinal walls and proliferate.

- Many probiotic combinations lack research. No studies exist showing their compatibility and experts agree too many different bacteria may be antagonistic to each other.
- The selection of bacteria for incorporation in probiotic supplements that are on the federal GRAS (Generally Recognized as Safe) list is imperative for the products safety.
- Fortification of probiotics with prebiotic fructooligosaccharides (FOS) enhances the value of the probiotic supplement by providing a nutrient that selectively enhances the growth of friendly cultures [68].

**Regulations and quality control of probiotic containing dietary supplements**

Regulations in only a few countries have made it possible to get an official approval of health claims— for example, in Sweden, the United Kingdom, and The Netherlands. With the acceptance of the new regulation from the European Union, the use of unauthorized claims and promises will be impossible. This may also lead to a swift move from general claims about health benefits and intestinal health to more-precise claims and products targeted to more specific areas of health [65]

When manufacturing probiotic products, high quality standards and processes are imperative. This ensures that the product is manufactured to the highest standard to result in a product that not only meets label specification but is also effective and safe to use. In addition to this, the product should be tested by an independent, fully accredited laboratory (lab) which has the expertise to enumerate (count) the probiotic strains. Quality assurance should rank highly on the priority list for each company and full traceability should be as standard [68].

In absence of any rigid or well-defined regulatory norms, manufacturers, consumers as well as the regulatory authorities are facing problems. It is obvious, that due to discrepancies in the health claims of probiotics and adoption of different regulations and methods of analysis by different countries around the world, establishment and enforcement of a globally accepted regulation and a quality assurance program for probiotics is essential to obtain consistent product [69].

Regulatory and labeling issues related to probiotics are complicated because they differ for each country [70] and status of probiotics as a component in food is currently not established on an international basis [71]. Problem of labeling still exists due to adoption of different regulations and methods of analysis by different countries around the world [72]. Probiotic foods have not been properly identified, documented, manufactured under Good Manufacturing Practices or proven clinically, which lead consumers in difficulties to decide and confirm whether they are adopting the reliable product [73]. Worldwide regulations related to probiotic foods are incoherent and assay methods are inconsistent, which resulted in existence of following problems related to labeling [72].

So to be practically useful to consumers, labels should have the following information: (a) notification of the presence of live bacteria; (b) the precise nature of the bacteria; (c) numbers of each species, in units comprehensible to consumers and microbiologically accurate; (d) the minimum amount necessary to bring about any claimed health effect, either in terms of numbers of bacteria or of servings; and (e) the accurate content at the time of purchase, not just at some stage during manufacture [66].

**Conclusion**

From the discussion presented in this review is clear, that a full traceability programme should be run and all raw material and finished product batches should be a subject to quality control analysis to ensure the best possible quality throughout the production cycle and for the whole shelf life of the product. It is also obvious, that the manufacturing of probiotic products of high quality standards and processes is imperative and adoption of different regulations and methods of analysis by different countries around the world is essential to obtain consistent safe products with high quality.

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