1. Introduction

Studies employing genes sequence for genotyping analysis of microorganisms, are allowing the knowledge expansion about the microbiota of the human gastrointestinal tract (GIT). Only in the last decade, the number of species detected molecularly has exceeded on a large scale the number of species accessible by cultivation-dependent methods.

The molecular techniques ranging from the identification of intestinal microbiota, particularly probiotic microorganism in different environments, detection of pathogenicity genes in foods, identification and quantification using real-time polymerase chain reaction (PCR), till studies with proteomics approach, which evaluate the expression of genes of interest or the changes in the host due to the microorganisms impact, have providing new perspectives in the investigation of diversity, abundance and dynamics of the intestinal ecosystem.

Research on probiotics microorganisms has focused on methods of evaluating the GIT microbiota survival and function, cross-talk between the intestinal microbiota and the host and the probiotic interactions with the immune system. Actually, the data generated by clinical studies reinforces the effect of this microbiota on the human health.

A substantial number of clinical studies have supported the idea that health can be affected by the daily consumption of probiotics. The exploitation of these data allows understanding the mechanisms by which probiotic microorganisms survive the passage through the GI tract to interact with the resident microbiota, and affect physiological functions in the host. Thus the probiotics have been extensively studied and commercially explored in many different products in the world.
2. The gastrointestinal microbiota

The human gastrointestinal tract (GIT) is composed of several connected organs that are involved in nutrient conversion and providing energy sources from the food absorbed. This complex system has a well-known anatomical architecture that is approximately 7 m long, comprising a 300 m² surface area in adults. From the mouth to the colon, there exists a complex microbiota consisting of facultative and strict anaerobes, including streptococci, bacteroides, lactobacilli and yeasts. The microbial community, inhabitants of these organs, is collectively called the gut microbiota and is composed of a myriad of microbial cells that outnumber the cells number of our body by a factor of at least 10. In addition, there is a great diversity of species, some of which have not yet been identified or cultured, and understanding the dynamics of this population is a challenge to the TGI ecologist (Zoetendal, et al., 2008).

However, the development of molecular biology since the discovery of polymerase chain reaction (PCR) by Mullins and Fallona (1996) up to the current approaches "omics", have focused on molecular characterization of specific environments such as GIT, as well as their interactions with probiotic bacteria. The knowledge of this microbiota that is underway has increased our understanding of the beneficial effects of probiotics on the human and animal health.

Prior to birth, humans develop in a sterile environment, the womb. However, the rupture of the membranes at delivery exposes the neonate to a wide variety of microorganisms, especially those that colonize the GIT, forming its microbiota. Over the course of human development, this microbiota undergoes variations according to the stages of life and related to the habits and habitats to which the individual is exposed (Isolauri et al., 2004, Tiihonen et al., 2010).

The most dramatic changes in the composition of the intestinal microbiota occur during childhood. During the first days of life, the microorganism population is unstable and tends to stabilize with breastfeeding or the intake of breast milk substitutes. The greatest change in this composition, however, occurs through weaning and the introduction of solid foods (Favier, et al., 2002). Throughout adulthood, the intestinal microorganisms are relatively stable; however, this stability is reduced in the elderly (Tiihonen et al., 2010). These changes can be attributed to dietary restrictions, changes in eating habits and the increased incidence of diseases and concomitant medication use, all of which are found with increasing age (Gill, et al., 2001, Tiihonen et al., 2010).

Early studies focused on the changes in the human intestinal microbiota, reporting the reduction of anaerobes and bifidobacteria and an increase of enterobacteria in the elderly (Mitsuoka, 1990). However, recent studies suggest a lower stability and increased diversity of the intestinal microbiota with advancing age (Hopkins and Macfarlane, 2002; Maukonen, et al., 2008, Tiihonen et al., 2010).

The human GIT has a very complex microbial ecosystem that is based on competition and symbiosis (Mackie et al., 1999) and consists of at least 400 to 500 different bacterial species, approximately $10^{13}$ cells (Ott et al., 2004; Zoetendal, et al., 2004; Zoetendal, et al., 2008). This population, have the composition which differs both along the gastrointestinal tract as along
the lumen to the mucosa (Tiihonen et al., 2010), is affected by several factors; some are determined by the interactions between genetic, environmental or disease factors to which the individual is exposed, the diet, the secretion of mucus, digestive enzymes and intestinal peristalsis. As a result, each individual has a unique characteristic microbiota (Isolauri, et al., 2004; Ley, et al., 2006).

The lack of bacteria in the upper GI tract (esophagus, stomach and duodenum) is related to the composition of the luminal medium (acid, bile and pancreatic secretions). In addition, the propulsive motor activity at the end of the ileum eliminates most of ingested microorganisms, preventing the stability of bacterial colonization in the lumen (Guarner and Malangelada, 2003). However, the lower portion of the GI tract, comprising the lower duodenum and small and large intestines, contains a complex and dynamic microbial ecosystem, with a high density of live bacteria reaching concentrations $10^{11}$-$10^{12}$ cells / g of luminal contents, which corresponds to 1.5 kg of microorganisms (Moore and Holdeman, 1974; Whitman et al., 1998; del Piano, 2006).

In this environment, the permanent organisms that colonize and grow in the place where they are found are considered to be autochthonous microbiota, whereas the non-native or transients are those that are vehicled by food, water and environmental components passing through the region (Ley, et al., 2006).

The TGI naturally has the function of protecting the body against pathogens and / or toxic metabolites. This protection is ensured by a number of factors, including saliva, gastric acids, peristalsis, mucus, intestinal proteolysis, intestinal microbiota balance and the epithelial membranes with intercellular junctional complexes (Ouwehand et al., 2002).

The intestinal mucosa forms an interface between the body and luminal environment, with the function of allowing the passage of nutrients and simultaneously acting as a barrier against microorganisms, toxins and other undesirable substances. The mucus produced by the goblet cells exerts this protective function; therefore, the barrier effect is guaranteed by the physical, chemical and functional epithelium integrity (Cencič and Langerholc, 2010).

The balance of the microbiota has been gaining special attention from the scientific community for years, and many studies indicate and confirm a close relationship between intestinal disbioses and microbial imbalance in addition to intestinal homeostasis and the maintenance of the equilibrium of the intestinal microbiota. Some microorganisms, particularly the probiotics, have great importance in maintaining this balance.

Although feces are the most available sample to investigate the intestinal microbiota, it is questionable how well the fecal microorganisms represent the intestinal microbiota, as they originate from the lumen and the distal colon. Indeed, the composition of intestinal microbiota is different in the lumen and the distal colon and throughout the TGI and mucosa. Moreover, the TGI has large species diversity and consists of known species and those that have not yet been cultured.

Thus, for more precise information on the gut microbial population, appropriate samples should be collected during endoscopies or surgical procedures; however, such invasive
procedures are rather unsuitable and rarely used in research. Moreover, the scarcity of information on the effects of anesthetics and disinfectants used in these procedures suggests the possibility that they may compromise the investigation (Isolauri et al., 2004, Ley, et al., 2006). Therefore, the approaches of studies on human intestinal microbiota are usually based on in vitro or animal models and in the evaluation of the fecal microbiota.

3. Probiotics and human health

Evidence derived from clinical and mechanistic studies indicate that the health benefits promoted by healthy lifestyle habits and the consumption of a balanced diet rich in bioactive ingredients are approaches that are increasingly attractive to the pharmaceuticals and food industries in addition to the general population.

Functional foods are defined as any substance or constituent of a food that, in addition to providing basic nutrition, promotes metabolic and / or physiological health benefits (Walker, et al., 2006). These foods are broadly grouped into conventional foods, bioactive substances and synthesized foods. In general, the term refers to a food that has been modified to become functional or that naturally contains bioactive compounds. Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods (Shah, 2007).

Thus, the probiotic microorganisms capable of promoting beneficial effects in a host for the production of bioactive compounds or the equilibrium of the intestinal tract are often associated with functional foods.

There is a long history of health claims concerning the beneficial effects of probiotic microorganisms in food, particularly lactic acid bacteria and bifidobacteria. Additionally, studies involving probiotic microorganisms have distinguished these microbes into different categories according to their mode of action, the aims of the administration of the probiotics and their mode of administration in addition to claims regarding legal regulations.

4. Probiotics: History and concepts

There is a long history of the beneficial effects that some microbes have on human health, with the effects of lactic acid bacteria, in particular, being the earliest record. In a Persian version of the Old Testament (Genesis 18:8), there is a statement that “Abraham owed his longevity to the consumption of sour milk.” In 76 BC, the Roman historian Plinius recommended the administration of fermented dairy products for the treatment of gastroenteritis (Bottazzi, 1983; Schrezenmeir and de Vrese, 2001). However, studies involving these organisms and their clinical effects in animals and humans are contemporary and are based on the production of beneficial substances and / or the promotion of a balance that favors the microbial host.

The concept of beneficial microorganisms has been attributed to Lactobacillus bulgaricus when, more than a century ago, Elie Metchnikoff (1905) emphasized the importance of
lactobacilli in the intestinal microbiota, providing the properties of health maintenance and longevity to the host. However, the term “probiotics” was proposed decades later by Lilly and Stillwell (1965) in reference to a substance secreted by protozoa in symbiosis. Parker (1974) first used the concept of combining the use of organisms or substances, as opposed to antibiotics, to contribute to the balance of intestinal microbiota. The term was later popularized by Fuller (1989) and defined as a probiotic food supplement based on live microorganisms with beneficial effects to the host in balancing the intestinal microbiota.

The term “probiotic” has been widely used, and according to research data, the general concept has experienced subtle changes. Schrezenmeyer and Vrese (2001) defined the term as a microorganism preparation or product containing viable microorganisms in sufficient numbers to change, through colonization, the host microbiota, thus promoting health benefits. Salminen and colleagues (1999) defined probiotics as microbial cell preparations (or components thereof), viable or inactive, with favorable effects on the health and welfare of the host. Clearly, the benefits must be evaluated in terms of the mechanisms and properly established and documented selection criteria.

Some authors also extend the action of probiotics to inactive cells and argue that both living and dead cells in probiotic products can produce beneficial biological responses (Havenaar et al., 1992; Adams, 2010). This approach will open new perspectives for research, for example, about the amount of cells needed and the proportion viable / non-viable cells required to obtain the desired effect. Furthermore, the use of inactivated probiotics has attractive advantages, such as consumption safety and the possibility of products with long shelf lives (Adams, 2010).

The WHO and FAO (World Health Organization and Food and Agriculture Organization of the United Nations) maintain the general concept that defines probiotics as live microorganisms that, when consumed in adequate amounts, confer benefits to the host (FAO / WHO, 2001). In Brazil, according to the currently enforced food legislation, the National Sanitary Surveillance Agency (ANVISA) has set forth that, to produce the claimed benefits of a probiotic food, the product should contain a minimum number of viable probiotic cells between \(10^8\) and \(10^9\) Colony-former unit (CFU) per day (BRAZIL, 2008).

However, the scientific community agrees that the effects of probiotic microorganisms can vary depending on the species, the quantity ingested and the physiologic characteristics of the host. Furthermore, the current evidence suggests that the probiotic effects are species and even strain specific (FAO/WHO 2002, Isolauri et al., 2004, Tiihonem et al., 2010).

Although the *Lactobacillus* and *Bifidobacterium* have been predominantly used as commercial probiotic; the market is not exclusive to these genera. In fact, is growing the number of probiotic foods available to the consumer. Based in scientific studies, the regulatory agencies worldwide have characterized a broader number of microorganisms as probiotics. Because the technologic and functional characteristics, these strains have been used in food and pharmaceutical industry (Table 1).
Table 1. Some microorganisms used as probiotic cultures in commercial products.

| Species                          | Strains                                      |
|---------------------------------|----------------------------------------------|
| *Bacillus lactis*               | DR10™                                        |
| *Bifidobacterium adolescentis*  | ATCC 15703, 94-BIM                           |
| *B. animalis* and subspecies *lactis* | BB-12™                                    |
| *B. breve*                      | Yakult™, BB-03                               |
| *B. bifidus*                    | BB-11™                                       |
| *B. b essentials*               | Danone™                                      |
| *B. infantis*                   | Shirota™, Immunitas™, 744,                   |
| *B. lactis*                     | Bb-02, Lafti™, DSM-B94, DR10™                |
| *B. laterosporus*               | CRL431                                       |
| *B. longum*                     | BB536, SBT2928, UCC 35624                   |
| *Lactobacillus acidophilus*     | LA-1™, La-5™, NCFM, DDS-1, SBT-2062, La-14™ |
| *L. casei*                      | Shirota™, LC™, DN114001™, Immunitas™        |
| *L. casei shirota*              | Yakult™                                      |
| *L. casei* ssp. *defensis*      | Danone™                                      |
| *L. lactis*                     | L1A,                                         |
| *L. fermentum*                  | RC-14                                        |
| *L. helveticus*                 | B02                                          |
| *L. johnsonii*                  | La1™                                         |
| *L. paracasei*                  | CRL 431™                                     |
| *L. plantarum*                  | 299 Probi™, LP115™, Lp01                     |
| *L. rhamnosus*                  | GG, GR-1, LB21, 271Probi™                    |
| *L. reuteri*                    | SD2112                                       |
| *L. salivarius*                 | Ls-33                                        |
| *Sacharomyces cerevisae*        |NCYC Sc 47                                    |
| *S. boulardii*                  | 17™                                          |

The characterization of the probiotic species or strain is supported by the screening of resistance to the adverse conditions in the TGI. To survive passage through the TGI, microbes must exhibit a resistance to a low pH, bile and pancreatic enzymes. Moreover, it is desirable that these bacteria display adhesion to the intestinal mucosa and pathogen exclusion abilities and have positive effects on the immune system of the host; evidently, these bacteria should be non-pathogenic and have a GRAS (Generally Recognized as Safe) status. These effects are evaluated by intensive *in vitro* and *in vivo* approaches. The intestinal homeostasis relies upon the equilibrium between substance absorption, secretion and the barrier capacity of the digestive epithelium, and probiotic microorganisms are highly related to homeostasis.

The scientific literature reports sufficient data to demonstrate that the benefits attributed to probiotics are inherent to their population increase in a given environment, concomitant with a decrease in potentially pathogenic bacteria (Jankovic et al., 2010). In addition, it had been demonstrated for more than 20 years that the intestinal microbiota of healthy individuals is altered with the ingestion of probiotics in favor of lactobacilli and bifidobacteria species. Although such alterations and the beneficial effects in healthy populations remains a complex issue (Saxelin, et al., 1993; de Vrese, et al., 2006), there is a
consensus on the association of disbioses with chronic inflammatory diseases (Manichanh, et al., 2006), obesity (Ley et al., 2006) and allergies (Penders et al., 2006).

There has been a substantial increase in the number of articles published in scientific journals and the lay press, focusing on the popularity of probiotic foods and their effects. Thus, the FAO and WHO (2001) established scientific committees, whose discussions have produced a document with guidelines designed to regulate the characterization of potentially probiotic microorganisms, ensure the security of the host, assess at the technological and commercial aspects of probiotics in food and evaluate the clinical proof of the expected effects on individuals (FAO / WHO, 2002).

Understanding the complex microbial system of the TGI will help to characterize the intestinal microbial community and recognize the mechanisms by which probiotics exert their effect on the health of humans and animals. Although the traditional culture-based and phenotypic techniques used to study this complex ecosystem are unfeasible, the current molecular approaches have increased our knowledge of the structure, diversity, interactions and mechanisms that influence the dynamics of the TGI microbial community.

5. Molecular approaches in the study of probiotic microorganisms

Studies of the gut microbiota that use traditional techniques for microbial cultivation are supported by phenotypic analysis based on morphological and biochemical characterization. These techniques are laborious, time consuming, subject to misinterpretation and identify only approximately 40% of the microbiota (Carey et al., 2007). The reasons for the deficiencies in microorganism cultivation by traditional methods include ignorance of the nutritional profile of the microorganism, culture medium selectivity, the stress imposed by cultivation procedures, the need to restrict the environmental conditions and difficulties in simulating the host interactions with microorganisms (Zoetendal, et al., 2004).

Research involving nucleic acid analysis indicated that the majority of the bacteria in a variety of ecosystems are different from those related on the cultivation methods. This idea led to the development and application of methods that are independent of the culture medium to study complex microbial ecosystems (Zoetendal, et al., 2004; Zoetendal, et al., 2008).

The polymerase chain reaction (PCR), developed by Kary Mullis in the 1980's, enabled the in vitro production of multiple copies of specific DNA sequences, without cloning (Alberts, et al. 1994). Variations of this technique have targeted the needs and advancement of biotechnology.

In addition, LAB and bifidobacteria have received much attention, especially since the creation of the consortium for sequencing the genome of these microorganisms (Lactic Acid Bacteria Genome Consortium - LABGC) in the U.S., which culminated in the genomic sequencing of industrial strains and many other relevant sequences that are ongoing. Currently, fourteen strains of *Lactobacillus* and ten strains of *Bifidobacterium* have been sequenced by the consortium (http://www.jgi.doe.gov/genome-projects/) or by private initiatives, such as *B. longum* NCC2705 in 2002, the first bifidobacteria to have its genome sequenced, and *L. plantarum* WCSF1 in 2003, the first *Lactobacillus* sequenced (O’Flaherty et al., 2009).
Molecular approaches to evaluate phylogeny and genetic and chemotaxonomic identification of the related species have been used successfully in the recent decades in studies. Additionally, the use of bioinformatics tools, along with access to available databases in the GenBank / NCBI (National Center for Biotechnology Information) has boosted research, aiming at the development of strategies for identifying target species (Costa, et al., 2011).

The significant increase in the knowledge of the structure, diversity and factors that influence the GIT microbial community dynamics and the mechanisms by which probiotics may influence intestinal homeostasis are due to ready access to their genomic data. Furthermore, the variety of in vivo immunoassays aimed at elucidating the physiological effects of probiotic therapies and the molecular approaches based on PCR, ribotyping and hybridization with probes have also contributed to the body of knowledge (Vaugh, et al., 2005; Walker, et al., 2006; Carey, et al., 2007).

Molecular markers are successfully employed in this environment favorable to the identification of probiotic microorganisms, and various molecular techniques have become powerful tools. Indeed, there are a large number of techniques that are useful for the identification of Lactobacillus in different environments (Moreira et al., 2005, , Costa, et al, 2011), the detection of pathogenicity genes in foods (Bottero, et al., 2004), the identification and quantification of bifidobacteria via real-time PCR (Masco, et al, 2007). In addition, proteomic approaches evaluates the expression of genes of interest or changes in the host related to the effects of the microorganisms (Yuan, et al. 2008; O'Flaherty, et al., 2010).

The use these of technologies associated with suitable choice of the molecular marker is very important to differentiate closely species. The recA gene has provided a high discriminatory ability for the differentiation of the LAB species (Figure 1).

Furthermore, studies employing the sequence analysis of genes for microorganism genotyping, such as ribosomal small subunit rRNA (SSU rRNA), allow the expansion of the knowledge about the diversity of the gut microbiota. Only a decade after the introduction of genotyping, the number of species molecularly detected in the TGI has greatly exceeded the number of species accessible using cultivation-dependent methods (Zoetendal, et al., 2008).

One of the most increasingly used techniques is real-time PCR or quantitative PCR (qPCR), which identifies and quantifies organisms of interest. This technique, coupled with the use of specific primers, has proven to be an accurate method that is suitable for the identification and quantification of microorganisms (Matsuki, et al., 2004). Moreover, this tool provides new perspectives in the studies of the diversity, abundance and dynamics of the intestinal ecosystem (Walker, et al, 2006; Masco, et al., 2007, Zoetendal, et al., 2008). Thus, the qPCR has attracted attention for being a reliable method that is highly sensitive for the detection and quantification of many organisms in different environments.

The technique is based on the traditional technology of PCR in combination with compounds that fluoresce at certain wavelengths, making it possible to monitor the amount of PCR products generated in each reaction cycle (Wittwer et al., 1997; Vitali, et al., 2003).
Figure 1. The phylogenetic tree consensus from the recA gene sequence comparisons, demonstrating the relationship of closely related species of the BAL, Bifidobacterium and enteric bacteria. The tree was constructed with the Neighbor-Joining method and the Clustal W algorithm. Genetic distances were computed by using Nei’s coefficient. Bootstrap values based on 1000 replicates are provided at branch nodes. The B. thuringiensis sequence was included as an out-group sequence.
The methods used for qPCR are based on the measurement of the fluorescence emitted as a function of the value of the cycle threshold (CT) or Crossing Point (CP), which is posteriorly related to mathematical expressions for absolute or relative quantification (Livak and Schmittgen, 2001; Pfaffl, 2001). The CT method is directly related to the quantity of the amplification product in the PCR reaction.

The normalization of the target gene using an endogenous standard is recommended (Pfaffl, 2001). The addition of a gene normalizer to the reaction is highly recommended and is intended to correct any concentration differences or defects in DNA extraction. Normalization ensures that fluctuations in the signal strength due to impurities or amounts of target DNA below the detection limit are taken into account during the analysis. However, the uniformity of the normalizer gene during the entire process or the stability of the expression during the experimental treatment must be confirmed (Kubista, et al., 2006; Marcelino, 2009; Hofstätter, et al., 2010; Dang and Sun 2011).

In the development of these methodologies, some alternatives have emerged to further refine the technique. Thus, the application of qPCR to quantify only viable cells (vqPCR) has eliminated one of the common criticisms in the quantification of probiotic microorganisms because qPCR does not distinguish between viable and non-viable cells.

The approach of vqPCR is based on the differentiation between viable cells and non-viable cells based on the membrane integrity. Theoretically, the selective dye used can only penetrate the permeable membranes of dead cells and intercalate extracellular DNA. The dye makes the DNA unavailable for amplification due to the presence of an azide group, present in such substances as ethidium monoazide (EMA) or propidium monoazide (PMA), which allows cross-links between the dye and DNA after the exposure to high-intensity visible light. The photolysis of these substances (EMA and PMA) converts the azide group into a highly reactive nitrene radicals, which can react with any organic molecule in its vicinity, including DNA, which then cannot be amplified by PCR (Varma, et al., 2007; Fitipaldi, et al., 2010).

Unquestionably, the use of genetic tools has accelerated the knowledge and understanding of the complexities found in the intestinal microbiota and their interactions. It is now possible to gain a better comprehension of the role of these organisms, including the accurate analysis of the functionality of probiotics and to obtain strains lacking one or more proteins (O’Flaherty and Klaenhammer, 2010). Furthermore, it is obvious that an understanding of the interactions through the cross-talk between the intestinal microbiota and its host would expand the knowledge of the relationship between microbiota and their effects on health.

There is an increasing tendency of probiotic studies to focus on metagenomics (Ventura, et al, 2009), which is, which is defined as the study of the collection of genomes of an ecosystem and can be used to study the phylogenetic, physical and functional properties of microbial communities. From the point of view of functional genomics, the application of these technologies provides a wealth of information and fosters research aiming at a better understanding of probiotic microorganisms and their effects.
6. Market prospects

The interest in functional foods is directly related to the growing appreciation of the quality of life and disease prevention because these foods affect specific functions or systems in the human body and are intended to complement basic nutrition (Shah, 2007). The food industry has developed a variety of new products containing active ingredients that promote consumer health.

The global market for functional foods generated US$ 32.07 billion in 2000 and US$ 68.39 billion in 2005; in 2010, the total surpassed US$ 150 billion and continues to expand (Granato et al., 2010). Latin America is considered an emerging market, and despite the general lack of nutritional knowledge by the population, Brazil and Mexico are potential trade markets for probiotics (Granato et al., 2010). The probiotic market in Latin America grew 32% per year between 2005 and 2007 (Crowley, 2008), and the annual sales growth rate of probiotic drinks and yogurts was 5% between 2006 and 2011 (Özer and Kirmaci, 2010).

Among the functional foods, dairy products with functional claims accounted for almost 43% of the world market between 2005 and 2010 (Özer and Kirmaci, 2010). In this scenario, the use of probiotic microorganisms in foods and pharmaceuticals had such an increase in the world market, that the sales reached $ 15 billion in 2007, amounted to $21.6 billion in 2010 with the prospect of more than $ 31.1 billion by 2015 (Agheyisi, 2011).

Following the same trend, the sales of foods with functional claims reached $ 500,000 in 2007, representing 1% of the total spending on food in Brazil (Cruz, et al, 2007; Granato, et al., 2010). According to Euromonitor International Consulting data released in 2010, the market for products for intestinal microbiota balance had a 60% growth in Brazil in five years, from R $ 57 million in 2004 to $ 92 million in 2009 (Revista Fator, 2011).

Over the last two decades, a substantial number of research studies have supported the idea that health can be affected by the daily consumption of probiotic foods (Heyman and Menárd, 2002), with clinical evidence demonstrating the actual effect of these organisms to the host. These data provide an understanding of the mechanisms by which probiotic microorganisms survive the passage through the GI tract to interact with the resident microbiota and affect physiological functions in the host. In addition, there is much investigation into both the classification of probiotic strains and the production technologies and regulation of the products.

To assess the impact of scientific research in the dissemination and consolidation of the benefits of probiotics in the diet, a search was conducted using three major scientific databases (Isi Web of Knowledge, Pub Med and Scopus). The search was restricted to two periods, and the key word “probiotic” in the title of the publication was used as a selection parameter. On average, there were 410 publications from 1991 to 2001, whereas 2406 records were found in the 2002 to 2011 period. According to the database Isi Web of Knowledge, in a period of ten years (2001 to 2011), 2686 publications were available in the database, documenting 791 patents, and 100 records are related to reviews; all of the other publications are related to primary literature.
Clearly, in a market in which product development should meet the needs of the consumer, it is important that scientific research does not neglect the technology and logistical aspects or the regulations of each country. The market will continue to grow as consumers maintain an interest in the products offered; however, the credibility of the product is based on its effects, which are often supported by scientific studies and the "know-how" from the manufacturer.

The majority of probiotic products on the market includes *Lactobacillus* and/or *Bifidobacterium* species but also yeasts; *Bacillus* and *Enterococcus* are common in these products. (Shah, 2007; Gaggia, et al.; 2010). Some probiotics marketed in food and pharmaceutical industries worldwide, the microorganisms involved, category of product, manufacturer and country from origin are listed in Table 2.

| Country     | Category                          | Commercial brand      | Manufacturer      | Probiotic                                      |
|-------------|-----------------------------------|-----------------------|-------------------|-----------------------------------------------|
| Australia   | Ingredient                        | Probiomics            | Bioxyne           | *L. fermentum VRI003 (PCC)*                  |
|             | Capsules                          | Floratil              | Merck             | *S. boulardii*                                |
|             | Sachet                            | Fiber Mais Flora      | Nestlé            | *Lactobacillus reuteri*                        |
|             |                                   | Activia               | Danone            | *B. animalis DN173010*                        |
|             |                                   | Actimel               | Danone            | *L. casei defensis*                           |
|             |                                   | Batavito              | Batavo            | *L. casei*                                    |
|             |                                   | Chamyto               | Nestlé            | *L. johnsonii/ L. helveticus*                 |
| Brazil      | Sachet                            | Danito                | Danone            | *L. casei*                                    |
|             |                                   | Leite fermentado      | Paulista          | *L. casei*                                    |
|             |                                   | Leite fermentado      | Parmalat          | *L. acidophilus/L. casei/ B. animalis subsp. lactis |
|             |                                   | Sofyl                 | Yakult            | *L. casei shirota*                            |
|             | Traditional yogurt or Drinking yogurt | Vigor club          | Vigor              | *L. acidophilus/L. casei*                      |
|             |                                   | Yakult                | Yakult            | *L. casei shirota*                            |
|             | Cheese                            | Equilibra             | Danubio           | *B. animalis*                                 |
|             |                                   | SanBIOS               | Coop. Santa Clara | *B. lactis*                                   |
| Canadian/USA| Capsules, Fermented Milk, Fermented soy and Fermented rice | Bio-K+ CL1285 | Bio-K+ International | *L. acidophilus CL1285 & L. casei LBC80R |
| France      | Fermented milk                    | DanActive             | Danon              | *L. casei DN-114 001 (“L. casei Immunitas”)    |
|             | Ingredient                        | Lacteol               | Laboratory Houdan | *L. acidophilus LB*                           |
| Country       | Category                        | Commercial brand          | Manufacturer       | Probiotic                                                                 |
|---------------|---------------------------------|---------------------------|--------------------|---------------------------------------------------------------------------|
| Japan         | Fermented milk                  | Yakult                    | Yakult             | L. casei Shirota B. breve strain Yakult                                    |
|               | Ingredient                      |                           | Morinaga Milk Industry Co Ltd | B. longum BB536                                                          |
| Sweden        | Juice                           | GoodBelly ProbiMage       | Probi              | L. plantarum 299v                                                        |
|               | Juice, Cultured Milk            | ProViva                   | Probi              | L. plantarum 299v                                                        |
|               | Juice                           | Bravo Friscus/ProbiFrisk  | Probi              | L. plantarum HEAL 9 & L. paracasei 8700                                   |
|               | Probiotic chewable tablets or drops | Protectis               | Biogaia            | L. reuteri ATCC 55730                                                    |
| Switzerland   | Fermented milk                  | LC1                       | Nestlé             | L. johnsonii Lj-1 same as NCC533                                          |
| UK            |                                 | Floralfit (Blend strains) |                    | L. acidophilus La14, L. casei Lc11, L. salivarius Ls-33, L. plantarum Lp115, L. rhamnosus, Lr-32, B. lactis Bl-04 & B. longum Bl05. |
| USA           | Capsules                        | Align                     | Procter & Gamble   | Bifidobacterium infantis 35624                                            |
|               | Supplement                      | GanedenBC                 | Ganeden Biotech    | Bacillus coagulans GBI-30, 6086                                            |
|               | Capsules                        | Florajen products         | American Lifeline, Inc | L. acidophilus, Bifidobacterium lactis & B. longum                        |
|               | Yogurt                          | Activia                   | Dannon             | B. animalis DN173 010                                                     |
|               | Dairy products                  | Frozen Kefir, Milk cultured kefir, Traditional kefir | Lifeway Foods Inc. | L. acidophilus, L. casei, L. lactis, L. rhamnosus, L. reuteri, B. brev, B. lactis, L. plantarum, B. longum, Leuconostoc cremoris, Sacharomyces florentinus & Streptococcus diacetylactis. |
| USA/Finland    | Supplements or Chewable for kids | Culturelle, Dannon Danimals | Valio and Dannon   | L. rhamnosus GG (“LGG”)                                                   |

Table 2. Foods and pharmaceuticals probiotics products, marketed worldwide, manufacturer and microorganism in use.
The probiotic market is constantly changing. Within this context, many innovations that direct studies and the functional microorganism market are being applied, and there are prospects of many other approaches because this branch of science is challenging.

What factors are predominant in the probiotics development? From the standpoint of marketing, the factors are a fully expanding open field, and the numbers reflect this scenario. From a scientific standpoint, many studies are aimed at the selection of strains with desirable and efficient characteristics, invoking the research of new effects and the elucidation of the mechanisms of action. The application of techniques for the functional genomics of probiotic bacteria certainly will accelerate the development of such products (de Vos, et al., 2004).

Furthermore, advances in the “genomic era” will increasingly be used to answer questions related to interactions between organisms. Molecular biology and its tools, the access to molecular databases, and the speed with which information is disclosed are essential for accurate identification of the benefits attributed to probiotics.

Most of the probiotic bacteria currently marketed were selected on basis on their technological properties, but not for their ability to confer health benefits. However, is evident that the use and development of novel technologies aiming products that meet the nutritional and physiological requirements desired by the target population is a priority among research and Industries. Additionally, the “feedback” among science, industry and the market is extremely important, and is desired that there is dynamism between these sectors.

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