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MINI-REVIEW

Characterization of the intestinal microbiota and its interaction with probiotics and health impacts

Carine Nunes de Almada[1] · **Caroline Nunes de Almada**[1] · **Rafael Chacon Ruiz Martinez**[1] · **Anderson de Souza Sant'Ana**[1]

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Abstract The gastrointestinal tract (GIT) is a dynamic microecosystem containing a diversified microbiota of about 500–1000 different microbial species. Humans depend on their intestinal microbiota to carry out vital functions, and thus, equilibrium among intestinal groups of microorganisms is essential. In this review article, the use of traditional and molecular methods is discussed for the characterization of the intestinal microbiota, as well as its interaction with probiotics and their effects on health. An improved knowledge on intestinal microbiota composition and diversity and how changes in this microecosystem can cause or are associated with diseases remains far from being completely understood. Therefore, a better understanding of the GIT microbial populations is crucial, which will certainly contribute to the development of new strategies for the prevention and/or treatment of several diseases. The manipulation of the GIT microbiota by probiotics consumption is an interesting approach to maintain and restore human health.

Keywords Gut · Microbiota · Diseases · Probiotics · Immune system

Introduction

Microorganisms colonize practically the whole surface of the human body exposed to the external environment, including the skin, oral cavity, respiratory and urogenital membranes, and the gastrointestinal tract (GIT) (Gerritsen et al. 2011). The GIT is a complex and dynamic microecosystem containing a high microbial diversity, estimated at 500–1000 microbial species, which are in equilibrium (Collado et al. 2009). These microorganisms can be permanent residents of the intestinal microbiota or transient, environmentally acquired, for example, by food consumption (Gerritsen et al. 2011).

The intestinal microbiota has an important role in the improvement of the bioavailability of nutrients and degradation of nondigestible components of the diet, production of new nutrients, removal of toxic compounds, metabolism of carbohydrates and proteins, intestinal barrier, protection against diseases, boost of the immune system, and the development, maturation, and maintenance of motor and sensory functions of the GIT (Guarner and Malagelada 2003; Barbara et al. 2005; Rajilic-Stojanovic 2013). Therefore, the intestinal microbiota can be recognized as an active organ (Collado et al. 2009). The GIT microbiota composition is not homogenous since it varies in terms of spatial and temporal perspectives (Fig. 1a–c) (Sekirov et al. 2010). Due to the presence of acids, pancreatic and bile secretions, oxygen gradients, and the ileum motor activity, a stable colonization of the stomach and duodenum is not easy to be reached for the majority of the microorganisms. As a result, the small intestine only houses a few species and a reduced number of microorganisms. The bacterial populations increase along the GIT, reaching the highest numbers and diversity in the colon. Apart from variations in the microbiota composition, the luminal microbial populations also differ in terms of the ratios between anaerobic and aerobic microorganisms, which are lower at the epithelial surface of the mucosa than at the intestinal lumen (O'Hara and Shanahan 2006; Espey 2013).

The microbial colonization of the human intestine starts at the moment of birth. During the first year of life, its composition is simple but greatly varies among individuals and over time. After 1 year of age, the intestinal microbiota of children

✉ Anderson de Souza Sant'Ana
and@unicamp.br

[1] Department of Food Science, Faculty of Food Engineering, University of Campinas, Rua Monteiro Lobato, 80, Cidade Universitária Zeferino Vaz, CEP: 13083-862 Campinas, SP, Brazil

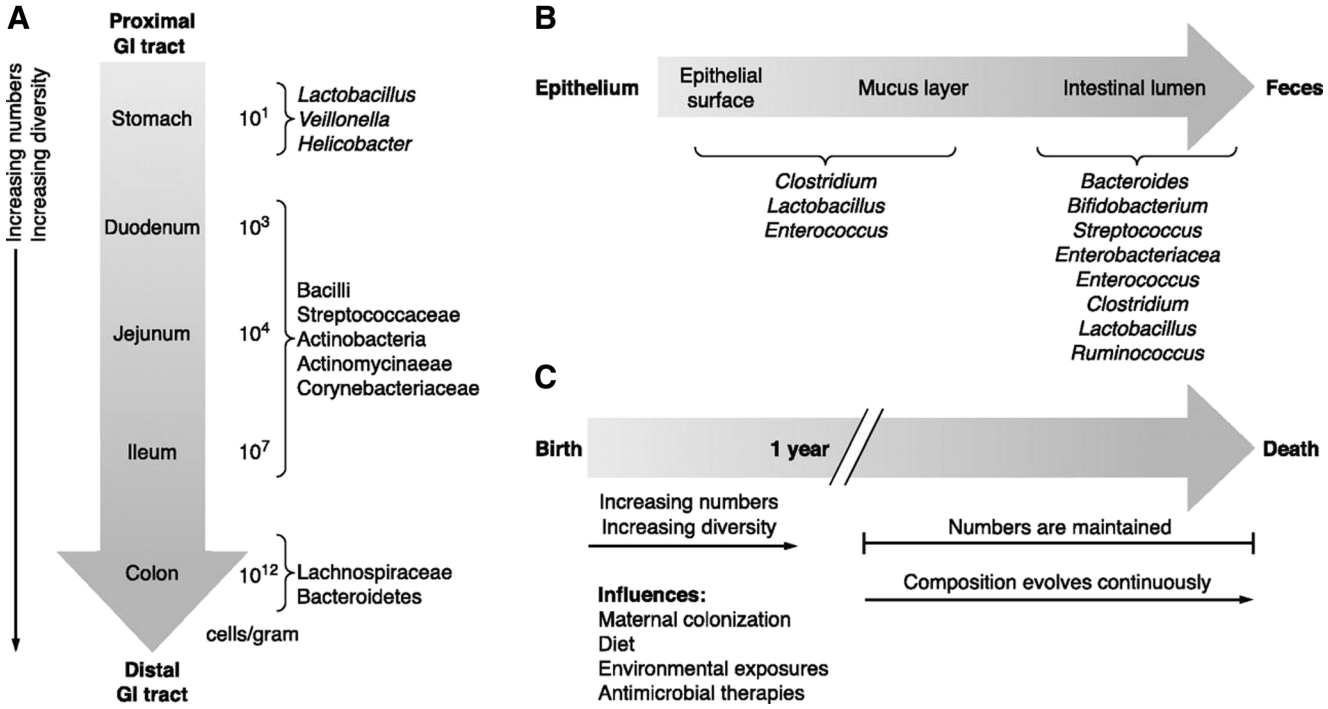

Fig. 1 Composition of the intestinal microbiota: spatial and temporal aspects. **a** Variations in microbial numbers and composition throughout the gastrointestinal (*GI*) tract. **b** Longitudinal variations in microbial composition in the intestine. **c** Temporal aspects of microbiota establishment and maintenance, and factors influencing microbial composition (Sekirov et al. 2010, with permission)

starts to become similar to that of a young adult and finally stabilizes (Fig. 1c) (Sekirov et al. 2010). The human GIT is sterile on birth and first colonized by fecal and vaginal microbiota acquired from the mother, which is affected by the type of delivery (natural or cesarean) and hygienic practices. Later, the intestinal microbiota is influenced by feeding habits and environmental microorganisms, being stable and unique for every individual throughout his/her adult life (Salminen et al. 2005).

Some factors can influence the host microbiota composition including the mother's microbiota composition, diet, environmental exposition, and use of antimicrobial therapies (Fig. 1c). Other aspects, including the host's inflammatory state and the genetic background, also impact the microbiota and, therefore, are able to contribute to the individual's health state; however, their exact roles remain mostly unknown (Gerritsen et al. 2011). Despite this multiplicity of factors, the composition of the human intestinal microbiota is stable, with main groups dominating the microecosystem, although variations in the proportions of these groups are common (Sekirov et al. 2010). The dominant genera in the microbiota of an adult human being are *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Lactobacillus*, *Fusobacterium*, and various Gram-positive cocci, while *Enterococcus* and *Enterobacteriaceae* are considered subdominant genera (Fig. 1a, b) (Guarner and Malagelada 2003). Thus, variations in the intestinal microbiota composition among individuals do not compromise the maintenance of an adequate function.

Normally, the microorganisms and their hosts have a symbiotic relationship, where the host offers a nutrient-rich environment, and on the other hand, the diversified intestinal microbiota exerts beneficial effects upon the host (Lutgendorff et al. 2008). However, the equilibrium among the intestinal microbial groups (nonpathogenic versus pathogenic microorganisms) is essential for health maintenance, and once disrupted, the relationship between host and microorganisms can culminate in a pathologic condition (Collado et al. 2009; Prakash et al. 2011) (Fig. 2). Perturbations of the microbiota composition, also known as dysbiosis (Gerritsen et al. 2011), have been associated with a greater risk for specific diseases, including chronic GIT inflammatory diseases (Joossens et al. 2011), diarrheas (Young and Schmidt 2004), irritable bowel syndrome (Maukonen et al. 2006; Malinen et al. 2010), allergies (Suzuki et al. 2007), diabetes (Wu et al. 2010), and obesity (Turnbaugh et al. 2006).

Functional foods not only satisfy hunger and provide the basic nutrients, but also improve the host's well-being (Vergari et al. 2010). Among them, foods with additional new nutrients or components, such as probiotics, are a highly profitable market niche for food industries because of their valuable health potential (Bigliardi and Galati 2013).

Given the above, it is comprehensible that probiotics consumption seems to be an interesting and feasible approach to modulate the intestinal microbiota and to maintain or restore human health (FAO/WHO 2002). Therefore, the goal of this review article is to discuss probiotics interactions with the

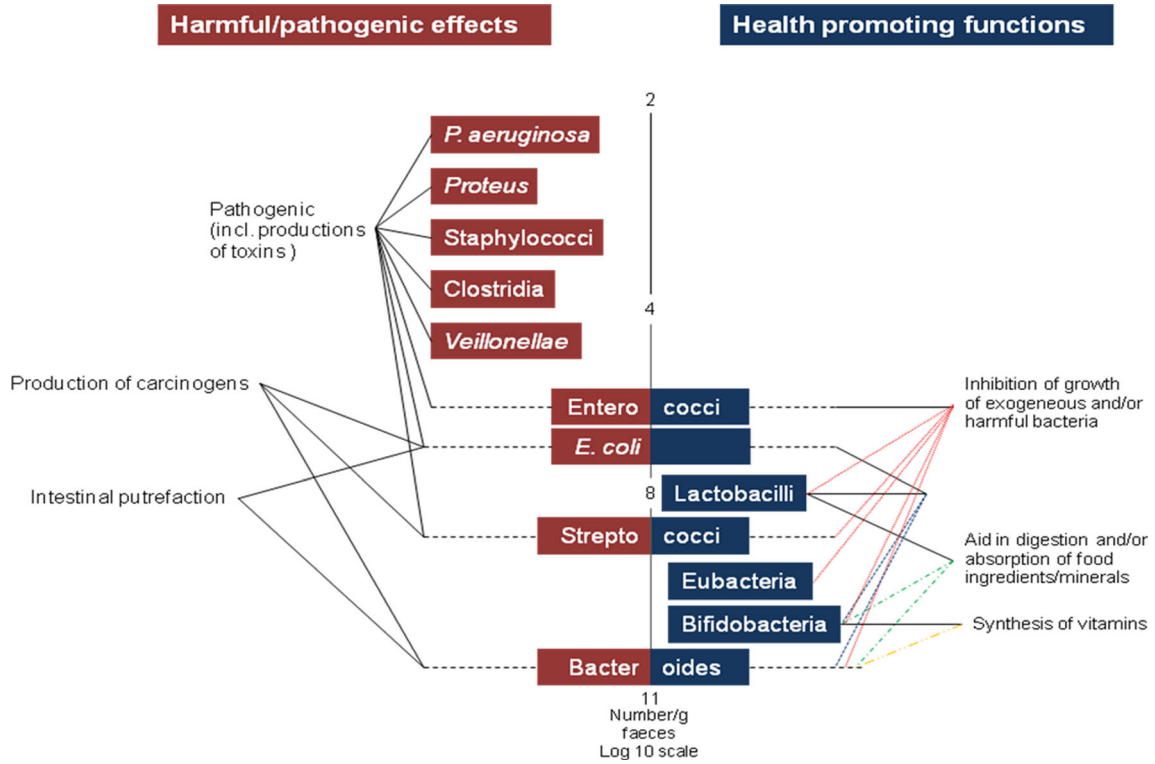

Fig. 2 The composition and health effects of predominant human fecal microbiota. Estimated numbers of genera present in feces are shown. Microorganisms can be divided into three groups based on their pathogenic potential or benefic effects on host: (i) bacteria that cause pathogenic effects, (ii) bacteria that exert beneficial effects, and (iii) bacteria that may lead to either harmful or beneficial effects (Gibson and Roberfroid 1995, with permission)

intestinal microbiota and their impacts on human health. Firstly, the techniques used to analyze the intestinal microbiota are presented, followed by an overview of the characteristics of probiotics and their action mechanisms. Finally, studies that correlate probiotics, the intestinal microbiota, and health interactions/impacts are discussed.

Techniques used to characterize the intestinal microbiota

The intestinal microbiota can be characterized according to its richness (number of species) and regularity (relative abundance of each species), representing its microbial diversity (Gerritsen et al. 2011). In Table 1, the main methods used for the characterization of the intestinal microbiota in the presence or not of probiotics are shown, which are discussed in detail in the succeeding subsections.

Culture-dependent method

This technique evaluates the microbiota composition by the use of different selective culture media for specific bacterial populations. Among the most widely used culture media for the evaluation of the main intestinal microbial populations that are worth mentioning are as follows: Wilkins-Chalgren agar for total anaerobic bacteria (Collado et al. 2007); de Man, Rogosa & Sharp (MRS) for total lactobacilli (Palomar et al. 2013); modified MRS for *Bifidobacterium* (Montesi et al. 2005); kanamycin-esculin agar for *Enterococcus* (Collado et al. 2007); Bacteroides bile esculin agar for *Bacteroides* (Carroll et al. 2010); reinforced clostridial agar (RCA) for *Clostridium* (Collado et al. 2007); and McConkey for enterobacteria (Palomar et al. 2013). Although culture-based methods for bacterial enumeration are highly reproducible, they are limited to distinguish between different bacterial groups. Despite this, it should be taken into account that the study of the intestinal microbial community is difficult since the majority of the microorganisms are strictly anaerobes. In addition, about 80 % of the intestinal microbiota cannot be cultivated under standard laboratory conditions (Eckburg et al. 2005).

The human GIT microbial diversity is markedly influenced by the approach used (Fig. 3). Firmicutes represents the most diverse phylotype both with respect to culture-dependent and culture-independent techniques. Bacteroidetes is a more diverse group when analyzed by molecular methods, in comparison to culture-based approaches. However, proteobacterial diversity has been better determined by the use of molecular techniques (Rajilic-Stojanovic et al. 2007).

Table 1 Methods used for characterization of the intestinal microbiota under different conditions

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
Culture-dependent	Feces	Assessment of the relationship between intestinal microbiota and the development of allergies in children[a]	–[b]	*S. aureus*, enterococci, lactobacilli, bifidobacteria, *Bacteroides* and clostridia	Björkstén et al. (2001)
Culture-dependent and FISH	Feces	Characterization of the intestinal microbiota composition as a prospective treatment target in infant atopic dermatitis[a]	–[b]	Culture-dependent (enterococci, *S. aureus*, streptococci, citrobacteria, enterobacteria, *E. coli*, *Klebsiella*, *Proteus mirabilis* and *C. albicans*); FISH (bifidobacteria, *Bacteroides*, lactobacilli/enterococci and *Clostridium histolyticum*)	Kirjavainen et al. (2001)
Culture-dependent, FISH and DGGE	Feces	Assessment of fecal microbiota of humans after consumption of dried milk containing probiotic (*L. rhamnosus* DR20)[a]	*L. rhamnosus* DR20	Lactobacilli, enterococci, enterobacteria, total bacteria, anaerobic microorganisms, total aerobic bacteria, bifidobacteria, clostridia, and *Bacteroides*	Tannock et al. (2000)
Culture-dependent and 16S rRNA sequencing	Feces and stomach and intestine contents	Comparison of fecal microbiota from healthy and autistic children[a]	–[b]	*Clostridium* spp. and *Ruminococcus* spp.	Finegold et al. (2002)
Culture-dependent	Feces	Characterization of fecal probiotic *Bacillus* (*B. cereus*, *B. clausii*, *B. pumilus*)[c]	*B. cereus* IP 5832, *B. clausii* and *B. pumilus*	–[f]	Duc et al. (2004)
Culture-dependent and DGGE	Feces	Comparison of composition and stability of intestinal microbiota of healthy individuals patients with irritable bowel syndrome[a]	–[b]	Culture-dependent (*Bacteroides*, bifidobacteria, lactobacilli, total aerobes, total anaerobes, coliforms and spore-forming bacteria); DGGE (*E. rectale*, *Eubacterium ventriosum*, *Ruminococcus*, *Clostridium* sp., *Clostridium glycolicum*, *Abiotrophia adiacens*, *Granulicatella elegans*, *Bifidobacterium* sp., and *Enterobacteriaceae*)	Mättö et al. (2005)
Culture-dependent	Large intestine	Evaluation of the effects of probiotic bacterium (*L. casei* CRL 431) on immune system[c]	*L. casei* CRL 431	Anaerobes, lactobacilli, and *Enterobacteriaciae*	Palomar et al. (2013)
Culture-dependent and qPCR	Feces and colonic mucosa	Comparison of fecal and mucosa-associated microbiota of healthy individuals and patients with irritable bowel syndrome[a]	–[b]	*Bacteroides*, *Clostridium,* bifidobacteria, *E. coli*, and *Lactobacillus*	Carroll et al. (2010)
Culture-dependent and FISH	Feces	Study of the differences between the composition of fecal microbiota in celiac and healthy children[a]	–[b]	Culture-dependent (total anaerobes, *Bacteroides*, *Clostridium*, *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Enterobacteriaceae*,	Collado et al. (2007)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
				Staphylococcus, and yeast); FISH (total microbial counts, *Bacteroides/Prevotella*, *C. histolyticum*, *Clostridium lituseburense*, *Eubacterium rectale-Clostridium coccoides*, *Bifidobacterium*, *Lactobacillus-Enterococcus*, *E. coli*, *Atopobium*, *Coriobacterium*, and sulfate-reducing bacteria)	
Culture-dependent and DGGE	Tissue and cecum content (first section of large intestine)	Evaluation of the effects of diet supplementation with prebiotic (fructooligosaccharides) or probiotic (*B. lactis* and *S. thermophilus*) in rats[d]	*B. lactis* BL and *S. thermophilus*	Culture-dependent (anaerobes, lactobacilli, clostridia, bifidobacteria, *Bacteroides*, and coliforms); DGGE (lactobacilli and *B. animalis* and *B. lactis*)	Montesi et al. (2005)
Culture-dependent and DGGE	Intestinal content	Study of the effect of probiotic bacterium (*Pediococcus acidilactici*) on intestinal microbiota and immune status of red tilapia (*Oreochromis niloticus*)[e]	*P. acidilactici*	Total aerobic and anaerobic bacteria, LAB	Ferguson et al. (2010)
Culture-dependent and DGGE	Feces	Assessment of the probiotic potential of *L. paracasei* A added to yogurt in children[a]	*L. paracasei* A	Culture-dependent (clostridia, bifidobacteria, total aerobes, enterococci, total anaerobes, *Bacteroides*, enterobacteria, lactobacilli); DGGE (*L. amylovorus*, *L. brevis*, *L. casei/paracasei*, *L. curvatus*, *L. bulgaricus*, *L. fermentum*, *L. fuchuensis*, *L. gasseri*, *L. helveticus*, *L. johnsonii*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. ruminis*, *L. sakei*, *L. salivarius*, *L. mesenteroides*, and *S. thermophilus*)	Marzotto et al. (2006)
Culture-dependent and FISH	Feces	Evaluation of probiotic bacteria supplementation (*B. lactis animalis* subsp. Bb-12) on intestinal microbiota of premature children (gestational age of 37 weeks)[a]	*B. lactis animalis* subsp. Bb-12	Culture-dependent (*Enterobacteriaceae*, *Enterococcus* and *Streptococcus* spp., *Staphylococcus* spp., anaerobic bacteria, *C. albicans*, *Clostridia*, *Bacteroides*, *Bifidobacterium* spp., and aerobic bacteria); FISH (total bacteria, *E. rectale*, *Bacteroides* and *Prevotella*, *Bifidobacterium* spp., *Lactobacillus* and *Enterococcus* spp., *Veillonellae*, *Streptococcus* and *Lactococcus* spp., *Enterobacteriaceae*, *Staphylococcus* spp., *C. histolyticum*, and	Mohan et al. (2006)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
				C. lituseburense)	
Culture-dependent and qPCR	Feces	Evaluation of a probiotic-fermented oat-based beverage (*B. longum* 46 and *B. longum* 2C) on modulation of microbiota in the elderly[a]	*B. longum* 46 and *B. longum* 2C	Culture-dependent (*Bifidobacterium*); qPCR (*B. adolescentis*, *B. animalis*/*lactis*, *B. bifidum*, *B. breve*, *B. catenulatum*, *B. dentium*, *B. infantis*, and *B. longum*)	Lahtinen et al. (2009)
Culture-dependent and TRFLP	Feces	Study of the effects of probiotics (*B. lactis* Bl-04, *B. lactis* Bi-07, *L. acidophilus* NCFM, *L. paracasei* Lpc-37, and *B. bifidum* Bb-02) on antibiotic-induced gut microbiota alterations[a]	*B. lactis* Bl-04, *B. lactis* Bi-07, *L. acidophilus* NCFM, *L. paracasei* Lpc-37, and *B. bifidum* Bb-02	Culture-dependent (*Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Clostridium*, and *Enterobacteriaceae*); TRFLP (*Bifidobacterium*, *Lactobacillus*, *Enterobacteriaceae*, *Bacteroides*, and *Prevotella*)	Engelbrektson et al. (2009)
Sequencing based on 16S rRNA (Sanger sequencing) and pyrosequencing	Feces	Evaluation of ciprofloxacine on human intestinal microbiota[a]	–[b]	Bifidobacteria, *Bacteroides*, *Parabacteroides*, *Alistipes*, *Oscillospira*, *Dialister*, *Clostridium*, *Dorea*, *Faecalibacterium*, *Subdoligranulum*, *Clostridiaceae*, *Eubacterium*, *Anaerostipes*, *Coprococcus* and *Lachnospira*, *Roseburia*, *Ruminococcus*, *Lachnospiraceae*, Clostridiales, Firmicutes, *Sutterella*, and *Akkermansia*	Dethlefsen et al. (2008)
DGGE and TRFLP	Feces and intestinal tissue	Study of the impact of probiotic administration (*L. casei* and *L. plantarum*) on endogenous microbial population[c]	*L. casei* and *L. plantarum*	Lactobacilli (*Lactobacillus* spp., *L. johnsonii*, *L. murinus*, *L. reuteri*, *L. gasseri*, *L. psittaci*, *L. plantarum*, and *L. helveticus*)	Fuentes et al. (2008)
DGGE and qPCR	Feces	Evaluation of prebiotic (galactooligosaccharide), probiotic (*B. animalis* Bb-12), and potentially probiotic (*Lactobacillus amylovorus* DSM 16698) on colonic human and pig microbiota[a, g]	*B. animalis* Bb-12 and *L. amylovorus* DSM 16698	DGGE (total lactobacilli, total bifidobacteria, and total bacteria); qPCR (*L. amylovorus*, total lactobacilli, total bifidobacteria, and total bacteria)	Martinez et al. (2013)
DGGE and qPCR	Feces	Evaluation of prebiotic (lactulose), probiotic (*S. boulardii*) and the symbiotic combination on human fecal microbiota[a]	*S. boulardii*	*Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Enterococcus*	Vanhoutte et al. (2006)
DGGE and qPCR	Feces	Qualitative and quantitative evaluation of the effects of yogurt on intestinal microbiota of healthy individuals[a]	–[b]	Lactic acid bacteria (LAB), *C. coccoides*, *C. perfringens*, and *Bacteroides*	García-Albiach et al. (2008)
DGGE	Feces	Study of galactooligosaccharide and/or probiotic (*B. lactis* Bb-12) effects on qualitative composition of *B. indigena* populations in human microbiota[a]	*B. lactis* Bb-12	*Bifidobacterium* (*B. adolescentis*, *B. ruminantium*, *B. angulatum*, *B. infantis*, *B. adolescentis*, and *B. pseudocatenulatum*)	Satokari et al. (2001)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
qPCR	Feces	Qualitative and quantitative determination of fecal microbiota in elderly and characterization of the impact of probiotic oat-based beverage on endogenous microbiota and the relation to inflammatory responses[a]	*B. longum* 2C (DSM 14579) and 46 (DSM 14583) and *B. lactis* Bb-12	*B. longum*, *B. adolescentis*, *B. bifidum*, *B. catenulatum*, *B. breve*, *B. animalis*, and *B. dentium*	Ouwehand et al. (2008)
FISH, microarray, and 454-pyrosequencing	Feces	Evaluation of the effect of probiotic bacteria (*L. reuteri* DSM 17938 and ATCC PTA 6475) on enterocytes migration and microbial diversity in the intestine[c]	*L. reuteri* DSM 17938 and ATCC PTA 6475	FISH and microarray (*L. reuteri*); 454-pyrosequencing (*L. murinus*, *Klebsiella*, *Staphylococcus*, *Parabacteroides*, *Bryantella*, *Lachnospiraceae*, *Enterobacteriaceae*, *Ruminococcaceae*, *Anaerotruncus*, and *Erysipelotrichaceae Incertae sedis*)	Preidis et al. (2012)
qPCR and 454-pyrosequencing	Feces	Study of the effects of probiotic (*B. lactis* DN-173 010) fermented milk consumption on intestinal inflammation[c]	*B. lactis* DN-173 010	qPCR (*Enterobacteriaceae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *B. lactis*, *L. lactis*, *L. bulgaricus*, and *S. thermophilus*); 454-pyrosequencing (*Bifidobacteriaceae*, *Porphyromonadacaeae*, *Prevotellaceae*, *Staphylococcaceae*, *Lachnospiraceae*, and *Lactobacillaceae*)	Veiga et al. (2010)
PCR and 454-pyrosequencing	Biopsy of mucosal tissue	Investigation of intestinal microbiota composition and characterization of the probiotic effects (mix VSL#3) in the mucosa of patients with irritable bowel syndrome[a]	*Lactobacillus* (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*), *Bifidobacterium* (*B. longum*, *B. breve*, *B. infantis*), and *S. salivarius* subsp. *thermophilus*	Firmicutes, Bacteroidetes, Synergistetes, Actinobacteria, and Cyanobacteria	Ng et al. (2013)
qPCR and FISH	Feces and biopsy of duodenal mucosa	Comparison of intestinal microbiota composition of healthy and sick individuals (irritable bowel syndrome)[a]	–[b]	qPCR (*B. adolescentis*, *B. bifidum* and *B. longum*, and *B. catenulatum)*; FISH (*Faecalibacterium prausnitzii*, *C. coccoides-E. rectale* group, *Bifidobacterium*, *Lactobacillus-Enterococcus* group, *C. histolyticum* group, *Bacteroides-Prevotella* group, *C. difficile*, *C. lituseburense* group)	Kerckhoffs et al. (2009)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
FISH	Feces	Investigation of the role of colonic microbiota on the development of lactose intolerance[a]	–[b]	*E. rectale*/*C. coccoides*, *Bacteroides*/*Prevotella*, bifidobacteria, and *Atopobium* group	Zhong et al. (2004)
FISH	Feces	Assessment of how the ingestion of different amounts of a probiotic (*L. johnsonii* La1) affects main bacterial populations in human fecal microbiota[a]	*L. johnsonii* La1	*E. rectale*, *Fusobacterium prausnitzii*, *Bacteroides*, *Atopobium*, *C. histolyticum*, *Bifidobacterium*, and *Lactobacillus*	Garrido et al. (2005)
TGGE and FISH	Feces	Assessment of the impacts of regular yogurt consumption on composition and metabolism of human intestinal microbiota[a]	–[b]	TGGE (*L. bulgaricus*, *L. casei*, and *L. mesenteroides*); FISH (*C. coccoides-Eubacterium rectale* group, *Clostridium leptum* subgroup, *Prevotella and Bacteroides*, *Bifidobacterium*, *Lactobacillus*, *Enterococccus*, *Weissella*, *Pediococcus*, *Vagococcus*, *Leuconostoc*, *Oenococcus*, *Streptococcus* and *Lactococcus*, *Atopobium* group and enteric group)	Alvaro et al. (2007)
DGGE	Feces	Evaluation of the therapeutical effects of probiotic yogurts in patients with irritable bowel syndrome (change of microbiota and relief of irritable bowel syndrome's symptoms)[a]	*B. lactis* Bb-12, *L. casei* 431, *L. acidophilus* La-5, and *L. fermentum* ME-3	*E. coli*, *C. difficile*, *L. rhamnosus* GG, and *Bifidobacterium*	Lee et al. (2013)
DGGE	Mucosa	Assessment of the effects of probiotic supplementation (*P. acidilactici*) on intestinal microbiota and relationship with growth performance of trout (*Oncorhynchus mykiss*)[e]	*P. acidilactici*	–[f]	Ramos et al. (2013)
DGGE and RISA	Feces	Characterization of intestinal microbiota of children[a]	–[b]	*B. bifidum*, *B. adolescentis*, *B. angulatum*, *B. catenulatum*, *B. longum* subsp. *longum*, *B. longum* subsp. *infantis*, *B. pseudolongum* subsp. *pseudolongum*, *B. breve*, and *B. gallicum*	Roger et al. (2010)
DGGE and RISA	Feces	Identification of bacterial colonization (*Bacteroides fragilis*, *C. leptum*, and *C. coccoides*) in patients with diseases such as cancer[a]	–[b]	*Bacteroides fragilis* group, *Clostridia leptum* subgroup, and *Clostridia coccoides* subgroup	Scanlan et al. (2008)
qPCR	Tissue (ileal mucosa)	Assessment of the impact of probiotic supplementation (*B. longum* AH1206) on health, growth, and development of neonatal pigs[g]	*B. longum* AH1206	Bifidobacteria	Herfel et al. (2013)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
qPCR and TRFLP	Feces	Assessment of bacterial community profile in fecal microbiota of healthy adult human after ingestion of probiotic yogurt containing *B. lactis* Bb-12 and *L. acidophilus* LA-5[a]	*B. lactis* Bb-12 and *L. acidophilus* LA-5	qPCR (*B. adolescentis*/*ruminantim*, *B. bifidum*, *B. breve*, *B. catenulatum* group, *B. longum* group, *Bacteroides-Prevotella* group, *Blautia coccoides* group, *C. leptum* group, Enterobacteriaceae, *Enterococcus*, and total bacteria); TRFLP (*Clostridium*, *Lachnospiraceae*, *Faecalibacterium*, *Blautia*, *Subdoligranulum*, *Enterococcus faecalis*, *Desulfovibrio*, *Eubacterium*, *Ruminococcaceae*, *Peptostreptococcaceae*, *Alistipes*, *Bacteroides*, *Parabacteroides*, *Prevotella*, *E. coli*, and *Blautia*)	Filteau et al. (2013)
TRFLP	Feces	Characterization of human intestinal microbiota variability[a]	–[b]	Total bacteria	Li et al. (2007)
qPCR	Feces	Identification and quantification of a probiotic bacterium (*L. rhamnosus* GG) in human fecal samples[a]	*L. rhamnosus* GG	*L. rhamnosus* Lc705, *L. casei* ATCC 334, *L. zeae* ATCC 393, *L. rhamnosus* ATCC 7469, *L. rhamnosus* 1⁄6, *L. rhamnosus* E-97800, *L. rhamnosus* VS 1020, *L. rhamnosus* VS 1019, and *L. rhamnosus* GG	Ahlroos and Tynkkynen (2009)
Metagenomics	Feces	Comparison of intestinal microbiomas of adults and children[a]	–[b]	*Bacteroides*, *Dorea*, *Enterococcus*, *Citrobacter*, *Eubacterium*, *Streptococcus*, *Propionilbacterium*, *Raoultella*, *Bifidobacterium*, *Parabacteroides*, *Salmonella*, *Ruminococcus*, *Collinsella*, *Enterobacter*, *Clostridium*, *Escherichia*, *Shigella*, and *Klebsiella*	Kurokawa et al. (2007)
FISH and metagenomics	Feces	Investigation of fecal microbiota in patients with Crohn's disease[a]	–[b]	Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria	Manichanh et al. (2006)
Metagenomics	Feces	Analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis[a]	–[b]	*Escherichia*, *Haemophilus*, *Clostridium*, *Klebsiella*, *Serratia*, *Veillonella*, *Enterobacter*, *Enterecoccus*, *Megasphaera*, *Pseudomona*, *Staphylococcus*, *Bacteroides*, *Shigella*, and *Gemella*	Wang et al. (2009)
Metaproteomics	Feces	Identification of microbial proteins in fecal samples to gain information about genes expressed and key microbial functions in human gut[a]	–[b]	–[f]	Verberkmoes et al. (2009)

Table 1 (continued)

Method	Source	Application	Probiotic microorganism (s) tested	Detected microbiota	References
Metatranscriptomics	Feces	Functional analysis of human intestinal microbiota[a]	–[b]	*Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, Prevotellaceae* and *Rickenellaceae*	Gosalbes et al. (2011)
Metabolomics	Blood	Effects of intestinal microbiota on blood metabolites[c]	–[b]	–[f]	Wikoff et al. (2009)
Microarray	Feces	Evaluation of the effect of probiotics-containing biscuit in the intestinal microbiota of the elderly[a]	*B. longum* Bar33 and *L. helveticus* Bar13	*Bacteroides-Prevotella*, *Clostridium* cluster, *Lactobacillaceae*, *Bifidobacteriaceae*, *Bacillaceae*, *Fusobacterium*, *Cyanobacteria*, Enterococcales, *Enterobacteriaceae*, and *Campylobacter*	Rampelli et al. (2013)
454-Pyrosequencing	Feces	Assessment of the effect of probiotic preparation in digestive health of cystic fibrosis patients[a]	*L. reuteri* DSM 17938	Fusobacteria, Bacteriodetes, Actinobacteridae, Firmicutes and Proteobacteria	Del Campo et al. (2014)
qPCR and 454-pyrosequencing	Fecal and cecal samples	Evaluation of the effect of host diet on the intestinal persistence and gene expression of *L. plantarum* WCFS1 in healthy and health-compromised[c]	*L. plantarum* WCFS1	*Lactobacillus*, *Ruminococcus*, *Lachnospiraceae*, *Bacteroides*, Bacteroidales, *Desulfovibrio*, *Desulfovibrionaceae*, and *Anaerotruncus*	Tachon et al. (2014)
qPCR and 454-pyrosequencing	Feces	Assessment of changes in the fecal microbiota of adults consuming probiotic (*L. casei* Zhang)[a]	*L. casei* Zhang	*Phascolarctobacterium*, *Bacteroides*, *Roseburia*, *Faecalibacterium*, *Lachnospiraceae*, *Bifidobacterium*, *Lactobacillus*, *Coprococcus*, *Subdoligranulum*, *Anaerostipes*, *Prevotella*, *Dorea*, and *Parasutterella*	Zhang et al. (2014)

Only some of the studies listed in this table were discussed in the manuscript text since it would not be feasible to handle all of them in a minireview; moreover, this was not the only purpose of the work. For further details, please go through the references provided in the table

DGGE denaturing gradient gel electrophoresis method, *FISH* fluorescent in situ hybridization method, *PCR* polymerase chain reaction, *qPCR* quantitative (real-time) polymerase chain reaction, *RISA* ribosomal intergenic spacer analysis, *TGGE* temperature gradient gel electrophoresis method, *TRFLP* terminal restriction fragment length polymorphism analysis

[a] Observed in humans

[b] In this study, the method was used only for the analysis of the intestinal microbiota (without administration of probiotics)

[c] Observed in mouse

[d] Observed in rats

[e] Observed in fish

[f] The intestinal microbiota was not fully described in the study

[g] Observed in pigs

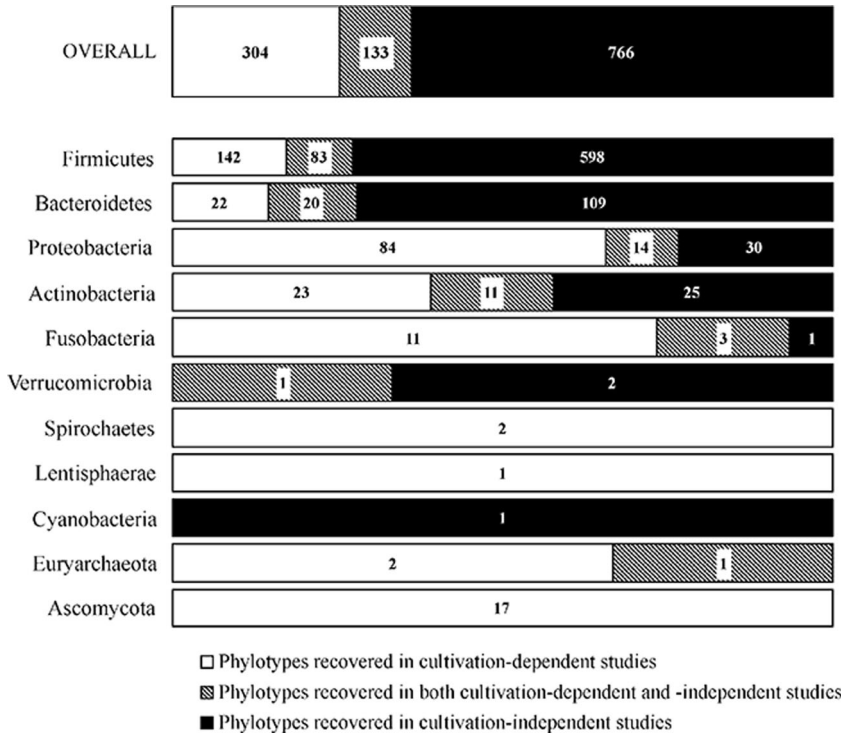

Fig. 3 Distribution of prokaryotic phylotypes in the GIT, shown overall and in terms of phylotypes (eight bacterial, one archaeal, and one eukaryal). The diversity of the gastrointestinal isolates that have been fully characterized, but lack the SSU rRNA gene sequence, was taken into account for construction of this figure (Rajilic-Stojanovic et al. 2007, with permission)

Sanger sequencing

Considering the limitations of culture-dependent methods, molecular techniques based on the analysis of the 16S ribosomal RNA (rRNA) bacterial gene, a marker of genetic diversity, have emerged. This gene was chosen because of its small size (1.5 kb) and due to the fact that it strikes an appropriate balance of conservation and variability, which enables the differentiation of species and strains but also the identification of members belonging to the same phylogenetic group (Peterson et al. 2008).

Sanger sequencing is used to analyze the microbial diversity as 16S rRNA gene sequences are resolved into operational taxonomic units (entities of taxonomic classification of group or individual species) based on their individual percent sequencing identity (%ID). Hence, specific %IDs are recognized as indicators of taxonomic resolutions, which indicate the species, genus, and family (Peterson et al. 2008). The technique has the advantages of presenting good resolution and sensitivity; however, its main limitations are the time required for its performance, low yield, high cost, and the need for an extensive data analysis (Delmont et al. 2012). The method was used by Finegold et al. (2002) to compare the intestinal microbiota in children with regressive autism and healthy ones. The authors observed that the number of clostridial species in the feces in the former group was greater than the one determined in the control group.

Pyrosequencing

Pyrosequencing is one of latest low-cost options that are replacing the need to sequence every 16S rRNA gene in its totality. The technique generates large numbers of 16S rDNA sequences by amplification of the variable regions selected on the inside of the 16S rRNA gene. Pyrosequencing is able to sequence a large amount of bases with good precision and yield (Margulies et al. 2005). In addition, the technique shows optimum sensitivity. Interestingly, pyrosequencing is able to determine the entire phylogenetic spectrum and allows taxonomic characterization and assessment of intestinal microbial populations at different taxonomic levels (Hooda et al. 2012). Several researchers have successfully used the method to evaluate the impact of probiotic consumption on the intestinal microbiota, as demonstrated by the studies published by Del Campo et al. (2014), Tachon et al. (2014), and Zhang et al. (2014).

DNA microarrays

DNA microarray technology is a powerful tool developed specifically for high-throughput screening of microbial communities. This methodology has the potential to provide information about the pathogenesis of several diseases—infectious and noninfectious ones (e.g., cancer) (Paul et al. 2007). Moreover, it exhibits an efficient cost-benefit relationship and good levels of sensitivity and selectivity and requires short time for performance of analysis (Heller 2002). Its main

drawbacks include the low detection limit and the inability to identify new species and strains (Sekirov et al. 2010). This technique was able to detect changes on the intestinal microbiota of elderly people that consumed probiotics-containing biscuits once a daily for a month (Rampelli et al. 2013). According to the authors, in individuals that ingested the probiotic, the age-related increase of the opportunistic pathogens was reverted, in comparison to individuals supplemented with placebo-containing biscuits.

Fluorescence in situ hybridization (FISH) and real-time quantitative polymerase chain reaction (qPCR)

FISH and qPCR are valuable tools to assess the intestinal microbiota. Furthermore, both techniques can be combined to confirm the results observed. Kerckhoffs et al. (2009) used FISH and qPCR techniques to evaluate the intestinal microbiota composition of healthy individuals and those diagnosed with irritable bowel syndrome. The authors observed a decrease in the bifidobacteria populations in the latter, in comparison to the levels determined in healthy subjects.

FISH uses oligonucleotide probes labeled with florescence, conceived to hybridize with unique 16S rRNA sequences present in specific microorganisms. One of the main drawbacks for FISH is the fact that only a few probes can be used per analysis (Zoetendal and Mackie 2005).

The qPCR is a precise and sensitive method for the enumeration of microorganisms in complex ecosystems. In this technique, specific or universal primers can be used and a standard curve is generated with the use of a reference strain, from which results will be derived for microorganisms' enumeration. An important limitation of both methods is seen in cases where no adequate cultivable strains are available for use as a reference strain. Furthermore, they do not allow the identification of new species since the primers used target a specific previously known bacterial taxonomic group (Prakash et al. 2011).

Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE)

DGGE and TGGE are basically forms of electrophoresis which use either a temperature or a chemical gradient, respectively, to denature samples as they move across an acrylamide gel. In reality, the samples analyzed by these methods correspond to DNA fragments previously extracted directly from the microbial community, without the need of pre-enrichment steps, and then amplified by the PCR method (Valásková and Baldrian 2009). According to their guanine and cytosine contents (GC), the PCR products will migrate in the acrylamide gel and a differential band pattern will be observed, which represents the sample microbial diversity. DGGE and TGGE allow a high number of samples to be screened simultaneously (McCartney 2002). The techniques can be used to analyze the whole community or specific populations or groups within a sample and have been successfully used to study the complexity and dynamics of human fecal microbiota (Alvaro et al. 2007; Lee et al. 2013). However, the main limitation of these methods is the detection of heteroduplex molecules, which can result in an overestimation of the real microbial community and, therefore, lead to inaccurate conclusions (McCartney 2002). Moreover, the PCR products are short and limited taxonomic information can be obtained. Both methods also have limited sensitivity and the reproducibility between gels is difficult (Muyzer 1999).

Terminal restriction fragment length polymorphism (TRFLP)

TRFLP is a powerful tool to evaluate the microbial diversity. TRFLP profiles are generated by the digestion of PCR-amplified 16S rRNA genes with a restriction endonuclease, originating a florescence-labeled terminal restriction fragment of variable length (Osborn et al. 2000). The fragments migrate distinctly in the gel electrophoresis, creating a specific band pattern for each sample. TRFLP is a useful quantitative technique for the evaluation of changes in the microbial populations and comparison of communities (Abdo et al. 2006). TRFLP is a fast, low-cost, and reproducible method (Smith et al. 2005); however, the TRFLP profile characterization is not easy since databases of TRF sizes may be imprecise (Kitts 2001). This technique was used by Filteau et al. (2013) to evaluate the impact of probiotic yogurt consumption on the fecal microbiota composition in healthy adults. According to the authors, no major differences were determined in the microbial profiles determined in fecal samples obtained from individuals fed with yogurt or placebo.

Ribosomal intergenic spacer analysis (RISA)

The RISA is a relatively new technique used for the analysis of the intestinal microbiota, as shown by Scanlan et al. (2008) and Roger et al. (2010). The method involves the PCR amplification of the intergenic space between the 16S and 23S rRNA genes (Feligini et al. 2015). It shows good resolution, detection limit, and reproducibility, besides being fast and inexpensive (Rastogi and Sani 2011); however, the application of the method can be limited by the absence of an available extensive database comprising the RIS taxonomy for intestinal microorganisms (Brown et al. 2005; Prakash et al. 2011). Nevertheless, the use of RIS sequences for the differentiation of microorganisms is known to be more useful than the analysis based on the 16S rRNA gene sequence. As an example, 16S rRNA gene sequences of species belonging to the genus *Bifidobacterium* can show 99 % similarity, whereas

RIS genetic sequences show greater divergence in the sequences of closely related species (Ventura et al. 2001).

Analyses focused on function—the "meta" family

Metagenomics

Metagenomics is a recent tool for the analysis of microbial communities. Indeed, it is a genomic analysis applied to all microorganisms present in a given microbial ecosystem, without prior identification. Metagenomics comprises culture-independent studies of the structures and functions of microbial communities and their interactions with the habitat (Lepage et al. 2013). The process is divided into two areas: (a) individual targets are amplified using the PCR method and the products sequenced—metagenomic study focused on a single gene; and (b) the total DNA is isolated from a sample and sequenced—random metagenomic study of all genes. The method provides a detailed survey of all genes that exist in a specific community (structure, composition, and function) in a single experiment (Gilbert and Dupont 2011). The technique provides information about the sequence of the microbiota genomes and can consequently be used to identify biological contributions and functions in this complex community, comparing healthy and ill individuals (Manichanh et al. 2006; Wang et al. 2009), as well as people of different ages (Kurokawa et al. 2007). The main disadvantage associated with the method includes the fact that the reads (one read is approximately equivalent to one gene size), mapped for reference genomes, are limited by the number of available sequenced genomes. This method depends on extensive bioinformatics analysis, and the cloning procedure is of great importance since it can directly affect the genetic information obtained (Sekirov et al. 2010).

Metatranscriptomics

Metatranscriptomics is another new genomic tool applied to the analysis of microbial communities and is based on the sequencing of nucleic acids extracted from microbial populations, as seen for metagenomics. While metagenomics deals with the evaluation of DNA sequences, metatranscriptomics involves the characterization of messenger RNA (mRNA), directly extracted from microbial populations (Gosalbes et al. 2011). Metatranscriptomics enables researches to understand how changes in the environment induce alterations in gene(s) expression in the whole community. Since RNA degradation is more likely to happen than expected for DNA, the sensitivity of metatranscriptomics depends on the number of read sequences obtained, which is the main disadvantage of the method and may result in relevant data loss (Sekirov et al. 2010). This technique can differentiate between expressed and nonexpressed genes. Thus, it focuses on the metabolically active members of a community (Su et al. 2012). Gosalbes et al. (2011) evaluated the gut microbiome and its functionality in health volunteers using metatranscriptomic analysis. According to the authors, the predominant families detected in the active microbiota included *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidaceae*, *Prevotellaceae*, and *Rickenellaceae* and were related to important functions such as carbohydrate metabolism, energy production, and synthesis of cellular components.

Metabolomics

Metabolomics provides an overall description of the metabolites present in a biological sample. The assessment of human metabolites can be an excellent indicator of a pathogenic process and also predict the effect of diet on human health (Russell and Duncan 2013). Wikoff et al. (2009) used metabolomics analysis and demonstrated an important relation between the intestinal microbiota and metabolite production, which were determined in plasma samples obtained from germ-free and conventional mice. According to the authors, the production of amino acid metabolites, including the antioxidant indole-3-propionic acid, and also organic acids containing phenyl groups was remarkably upregulated by the presence of gut microbes.

Metabolomics methods are considered to be faster and cheaper than the ones used for metagenomics studies. In addition, the method is very selective and sensitive (Dunn et al. 2005). However, one major limitation of metabolomics is that given the high complexity of the majority of tissues and fluids in the body, an overall view of all the metabolites is not feasible. Thus, not all metabolites are detected in heterogeneous natural environments and the presence of interfering compounds may burden the determination of their exact origin (Sekirov et al. 2010).

Metaproteomics

Although metatranscriptomics provides data about the genetic expression and activity, additional levels of cell localization and regulation occur at the protein level. Thus, the data obtained from the transcriptome and proteome can be substantially different. By the use of metaproteomics, the proteins are extracted from samples of mixed microbial populations, fractionated, separated by the use of the liquid chromatography method, and detected by the use of mass spectroscopy techniques (Langley et al. 2013). Metaproteomics has been used to study the main microbial functions in the intestine, including the diversity and abundance of the proteins in this organ, known as metaproteome. It is less expensive and faster than the metagenomic method(s) (Verberkmoes et al. 2009).

However, it is worth mentioning that the suitable protein fraction is difficult to be extracted and estimated (Su et al. 2012).

Probiotics and their mechanisms of action in the GIT

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Throughout the years, foods have been used as vehicles for probiotics delivery. The reasons for the successful application of foods as probiotic carriers include the following: (i) the positive health image of most foods; (ii) the fact that some foods, such as the fermented ones, contain live microorganisms, making the addition of probiotics easily acceptable by consumers; (iii) the ability of several foods to deliver probiotic microorganisms with good technological properties, which means that they are able to remain viable and physiologically active even at the end of their shelf-lives and do not negatively impact the food sensory attributes; (iv) the ability of foods to improve probiotics survival throughout the GIT; and (v) the fact that some foods provide bioactive food compounds, which can synergistically interact with probiotics and improve their benefits on the host health (Heller 2001; Da Cruz et al. 2010; Ranadheera et al. 2010; Staliano et al. 2015).

The main action mechanisms of probiotics are related to the GIT (Howarth and Wang 2013). Probiotics consumption seems to be an interesting and feasible approach to modulate the intestinal microbiota and to maintain or restore human health (FAO/WHO 2002). As previously shown (item 2), a wider knowledge on the intestinal microbiota composition and activity may facilitate the identification of microorganisms associated with different diseases and, hence, guide the development of probiotics able to precisely act in this microecosystem (Gueimonde and Collado 2012). In this sense, perturbations in the intestinal microbiota associated with intestinal disorders could be adequately prevented/treated by the use of selected probiotic strains (Bull-Otterson et al. 2013).

The main probiotic microorganisms include species belonging to the genera *Lactobacillus* and *Bifidobacterium*, although strains within few species of *Lactococcus*, *Streptococcus*, *Enterococcus*, *Saccharomyces*, *Bacillus*, *Brevibacillus*, and *Sporolactobacillus* have also been reported as probiotics (Borchers et al. 2009).

Probiotic microorganisms present certain characteristics related to their origin source, as well as regarding their physiology. These features include isolation from humans (not restricted to), resistance to a certain extent to food processing, and ability to adhere to the epithelial cells and to persist in the GIT. Furthermore, probiotics should exert benefit(s) on consumer health and not have pathogenic/virulence traits or toxic properties (Sanders et al. 2007; Nogueira and Gonçalves 2011). Since the beneficial effects of probiotic microorganisms are known to be strain-specific, they cannot be extrapolated to another strain or mixture of strains. Mixed probiotic cultures have the advantage of showing both the properties of the individual strains and the synergetic effects, thus increasing their overall efficiency. However, a study published by Almeida et al. (2008) suggested an antagonistic effect between the strains *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* in probiotic *açaí* yogurt, in which the growth of the latter was inhibited by an excessive production of hydrogen peroxide by *L. bulgaricus*. Therefore, inappropriate mixtures of probiotic strains may also result in reduced/loss health effects (Christensen et al. 2002; Gerritsen et al. 2011).

The main action mechanisms of probiotics include epithelial barrier function enhancement, improved adhesion to intestinal cells and pathogen inhibition by competition for adhesion sites, production of antimicrobial substances, and modulation of the immune system (Fig. 4) (Rijkers et al. 2010). Together, these mechanisms are able to modulate the composition of the intestinal microbiota and to prevent the growth of pathogenic bacteria as further detailed in the succeeding subsections.

Strengthening of the epithelial barrier

The main functions of the intestinal barrier are the maintenance of the epithelial integrity and protection of the host (Bermudez-Brito et al. 2012). The intestinal barrier defense mechanisms include the mucous layer, antimicrobial peptides, secretion of IgA, and the epithelial tight junctions (Ohland and Macnaughton 2010). Once the epithelial barrier is damaged, pathogenic microorganisms can lead to intestinal disorders, including inflammatory diseases (Hooper et al. 2001, 2003; Sartor 2006). Probiotics are important to maintain the integrity of this barrier and also to repair its damages (Bermudez-Brito et al. 2012). Probiotics can also increase the expression of genes that codify junction proteins and promote the mucous secretion (mucins) to improve the barrier function and to exclude pathogens (Anderson et al. 2010).

Increased adhesion to the intestinal mucosa and competitive exclusion of pathogenic microorganisms

Adhesion to the intestinal mucosa is an important property for the interaction between the probiotic microorganism and the host. Other features are also important, including the ability of probiotic microorganisms to modulate the immune system and to adhere to the intestinal mucosa (Collado et al. 2009).

Probiotics prevent the binding of pathogens to the intestinal cells by competitive exclusion for nutrients and adhesion sites in the mucosa (Collado et al. 2005; Bermudez-Brito et al. 2012; González-Rodríguez et al. 2012) and also by promoting

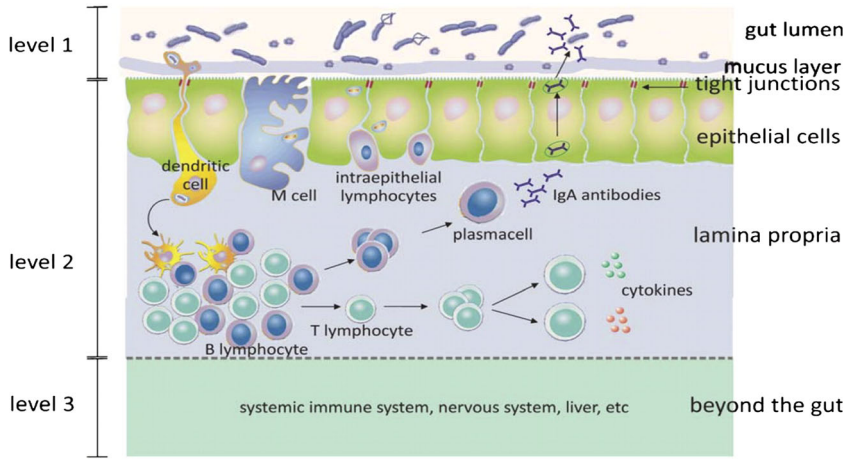

Fig. 4 Main mechanisms of probiotics action. Three levels of known or potential actions are presented. *Level 1*: Interference in the growth or survival of pathogenic microorganisms in the gut lumen; *level 2*: improvement of the mucosal barrier function and mucosal immune system; *level 3*: effect on the systemic immune system, as well as other cell and organ systems, including the liver and brain (Rijkers et al. 2010, with permission)

qualitative alterations in the intestinal mucin (Kim and Ho 2010).

Overall, probiotics are able to suppress the growth of pathogenic or potentially pathogenic microorganisms by the creation of a hostile microenvironment, elimination of available bacterial receptor sites, depletion of essential nutrients, and production/secretion of antimicrobial substances (Rolfe 1991).

Production of antimicrobial substances

Low molecular weight compounds such as organic acids and proteinaceous antimicrobial substances, known as bacteriocins, are produced by some probiotic strains (Bermudez-Brito et al. 2012). Organic acids such as acetic and lactic acid show good inhibitory effects against pathogenic microorganisms (Alakomi et al. 2000; De Keersmaecker et al. 2006; Makras et al. 2006). The bactericidal mechanism mediated by bacteriocins involves the destruction of the target cell by membrane pore formation or by the inhibition of cell wall synthesis (Nielsen et al. 2010; Hassan et al. 2012). Many lactic acid bacteria (LAB) also produce small antimicrobial proteins (AMPs) that can act against foodborne pathogens.

Probiotic bacteria are able to produce conjugated bile acids (bile salt derivatives) that have strong antimicrobial activity, and some probiotic strains produce metabolites that are able to inhibit the growth of fungi and Gram-negative bacteria (Bermudez-Brito et al. 2012).

Probiotics and the immune system

The immune system comprises the innate immune and the adaptive response. The innate system is the first line of defense and acts in a nonspecific way, which includes neutrophils, eosinophils, basophils, monocytes, dendritic cells, natural killer cells, and soluble factors. On the other hand, the adaptive system responds to antigens in a specific way and consists of T and B lymphocytes, humoral factors, and immunoglobulins. The innate and adaptive immunity work in collaboration (Tsai et al. 2012). The immune system activation may occur due to the competition for nutrients and colonization sites, antimicrobials production, and changes in the intestinal pH, among others. However, in most cases, the immune response is initiated by unknown factors (Shah 2007).

The host cells that most interact with probiotics are both the intestinal epithelial cells (IECs) and the dendrite cells (DCs) (Bermudez-Brito et al. 2012). The IECs and DCs can interact and respond to intestinal microorganisms through their pattern recognition receptors, which bind pathogen-associated molecular patterns present in the majority of pathogens, modulating the intestinal immune system (Gómez-Llorente et al. 2010; Lebeer et al. 2010). Furthermore, probiotic bacteria can also exert immune-modulating effects by interaction with monocytes/macrophages and lymphocytes (Bermudez-Brito et al. 2012).

Probiotics, intestinal microbiota, and health

The intestinal microbiota exerts an important role on human health and disease. The manipulation of these microorganisms by probiotics intake is an attractive approach, since they act by modulating the intestinal microbiota and can contribute with health maintenance and restoration (Gerritsen et al. 2011). A study performed by Sun et al. (2011) showed that *Bacillus pumilus* SE5, administered to fish as a dietary supplement, was capable to modulate the intestinal microbiota. It is believed that probiotics can act in three ways: (a) directly within the GIT, (b) interact directly with the mucous layer and the intestinal epithelium, and (c) outside the GIT, by impacting the immune system and other sites (Rijkers et al. 2010).

The mechanisms by which probiotic microorganisms are able to alter the intestinal microbiota include the reduction of the luminal pH, competition for nutrients, secretion of antimicrobial compounds (organic acids, biosurfactants, hydrogen peroxide, and bacteriocins, among others), prevention of both bacterial adhesion and invasion of the epithelial cells, and induction of antimicrobial compound production by the host (Fooks and Gibson 2002; Ng et al. 2009; Gerritsen et al. 2011). Table 2 lists some of the beneficial effects of probiotics in the GIT and at other sites; below, we further characterize these potential uses.

Inflammatory bowel diseases

Inflammatory bowel diseases include ulcerative colitis and Crohn's disease. Crohn's disease is characterized by an unequal inflammation that can affect any part of the GIT, whereas ulcerative colitis is a chronic inflammatory condition which only involves the large intestine (Prakash et al. 2011). Im et al. (2009) showed that *Bacillus polyfermenticus*, administered to rats, reduced the mortality rates, the seriousness of the colitis (according to weight loss, diarrhea, and mucosal damage occurrence), and the expression of inflammatory molecules (e.g., tumor necrosis factor alpha). The authors also reported that by the probiotic administration, apoptosis was overcome both in vivo and in vitro and a proliferation of epithelial cells was observed. Although not fully elucidated, the possible mechanisms involved could include the secretion of components that inhibit NF-κB activation and IL-8 secretion and induction of large amounts of IL-10 and low levels of IL-12 (Miquel et al. 2013).

The irritable bowel syndrome

The irritable bowel syndrome is characterized by abdominal pain, swelling, and alterations in the intestinal habits in the absence of any abnormality of the mucous. The disease is associated with abnormal intestinal communities, but their importance in the pathogenesis of the syndrome is not clear (Prakash et al. 2011). According to Dolin (2009) and Hun (2009), capsules containing *Bacillus coagulans* strains GBI-30 and 6086 were administered to patients and improved the symptoms of the irritable bowel syndrome, such as swelling and abdominal pain. A study published by Nobaek et al. (2000) reported that patients diagnosed with the irritable bowel disease that received, for 4 weeks, a supplement containing *Lactobacillus plantarum* DSM 9843a showed alleviation of pain and less flatulence. Malted milk containing *Bifidobacterium infantis* 35624 also alleviated the symptoms of the irritable bowel syndrome when administered to subjects. This response was associated with a normalization of the levels of anti-inflammatory and proinflammatory cytokines, suggesting an immune-modulating role for the microorganism (O'Mahony et al. 2005). A supplement containing *Lactobacillus rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *Bifidobacterium animalis* ssp. *lactis* Bb-12 was able to stabilize the intestinal microbiota and reduce the symptoms of the irritable bowel syndrome in patients, reducing the distension and abdominal pains (Kajander et al. 2008). Finally, a treatment using probiotic mix VSL#3 (*Lactobacillus casei*, *L. plantarum*, *L. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Bifidobacterium longum*, *Bifidobacterium breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*) during 4 weeks in patients with irritable bowel syndrome improved the symptoms of the disease. The authors associated the positive outcome with the modulation of the gut microbiota, as seen by the reduction of microorganisms' population belonging to the genus *Bacteroides* (Ng et al. 2013).

Lactose intolerance

Lactose is a sugar found in milk, which can be broken into glucose and galactose by the action of the lactase enzyme, mainly produced by children and some adults. However, some humans stop producing the enzyme in infancy, and if these individuals consume dairy products containing lactose, they can develop gastrointestinal symptoms such as abdominal distension, pain, flatulence, and diarrhea (Sanders et al. 2007). The use of probiotics to alleviate the symptoms of lactose intolerance is common due to the improvement in lactose digestion associated with the microorganisms (Maathuis et al. 2010). A study performed by He et al. (2008) suggested that the consumption of yogurt enriched with *B. animalis* for 2 weeks modified the composition of the intestinal microbiota, which could had been responsible, at some extent, for the alleviation of lactose intolerance symptoms in intolerant subjects. The consumption of a probiotic product containing *L. casei* Shirota and *B. breve* Yakult improved the lactose digestibility in patients with lactose intolerance. This positive observation could be related to an increase in the microbiota β-galactosidase activity and changes in the gut microbiota composition (Almeida et al. 2012).

Metabolic diseases

Obesity is a complex syndrome that develops from a prolonged disequilibrium in the energetic balance between caloric ingestion and expenditure (Guinane and Cotter 2013). Environmental and genetic factors influence obesity, and intestinal dysbiosis can also contribute to its development, since bacteria present in the intestinal microbiota influence nutrient uptake and energy regulation (Tilg et al. 2009; Prakash et al. 2011). Lines of evidence have shown that changes in the balance of the intestinal microbiota are associated with the development of metabolic diseases, including obesity, diabetes, hypercholesterolemia, and high blood pressure (Ebel et al. 2014).

Table 2 Selected beneficial effects of probiotics

Microorganism/product	Observed effect	References
B. polyfermenticus	Improvement of colon inflammation (mechanism of suppression of apoptosis and proliferation and migration in epithelial cells)	Im et al. (2009)
B. angulatum DSM 20098	Decrease in cholesterol levels	Al-Saleh et al. (2006)
B. infantis DSM 20088	Decrease in cholesterol levels	Al-Saleh et al. (2006)
Fermented cereal containing *L. casei* NCDC-19	Decrease in total cholesterol levels	Sindhu and Khetarpaul (2003)
Ganeden BC^{30} (*B. coagulans* GBI-30, 6086)	Reduction of evacuation in patients with irritable bowel syndrome	Dolin (2009)
		Hun (2009)
	Improvement of abdominal pain and swelling (symptoms of irritable bowel syndrome)	Maathuis et al. (2010)
	Relief of lactose intolerance symptoms	Kimmel et al. (2010)
	Improvement of immune system	Jensen et al. (2010)
	Anti-inflammatory effects and immune modulation	
Yogurt containing *B. animalis* DN-173010	Relief of lactose intolerance symptoms	He et al. (2008)
Probiotic product (*L. casei* Shirota and *B. breve* Yakult)	Improvement of lactose digestibility in patients with lactose intolerance	Almeida et al. (2012)
Yogurt containing *L. acidophilus* and *B. lactis*	Decrease in cholesterol levels	Ataie-Jafari et al. (2009)
L. reuteri	Reduction of gingivitis and dental plaque	Krasse et al. (2005)
L. rhamnosus JB-1	Reduction of symptoms of anxiety and depression	Bravo et al. (2011)
Fermented milk containing *L. casei* and *L. plantarum*	Reduction of symptoms and severity of infection caused by *E. coli*	Mirzaei et al. (2012)
Fermented milk containing *L. helveticus* LBK 16H	Reduction of blood pressure	Seppo et al. (2003)
Fermented milk containing *L. helveticus*		Jauhiainen et al. (2005)
Fermented milk containing *L. helveticus* IDCC3801	Prevention and relief of Alzheimer disease and other memory dysfunctions	Yeon et al.(2010)
Fermented milk containing *L. paracasei*-33	Improvement of allergic rhinitis symptoms	Wang et al. (2004)
Malted milk containing *B. infantis* 35624	Relief in symptoms of irritable bowel syndrome and immunomodulatory effects	O'Mahony et al. (2005)
L. reuteri	Improvement of periodontitis symptoms	Teughels et al. (2013)
Malt containing *L. mesenteroides* and *B. subtilis natto* RG4365	Improvement of *V. cholerae* inhibition by combination of two probiotic strains	Vidyalaxme et al. (2012)
Cheese containing *B. bifidum* A12, *L. acidophilus* A9, and *L. paracasei* A13	Immunomodulatory effects in intestine	Medici et al. (2004)
L. rhamnosus GG	Influence in the innate immunity and reduction in the proinflammatory cytokines expression, tumor necrosis factor-α and IL-6, consequently reduction in the inflammatory response	Amit-Romach et al. (2010)
B. breve M-16 V	Attenuation of allergic symptoms in allergic asthma-induced mice	Hougee et al. (2010)
L. plantarum 06CC2	Relief of influenza symptoms in mice	Takeda et al. (2011)
L. brevis CD2	Inhibition of periodental inflammation	Maekawa and Hajishengallis (2014)
S. thermophilus DSM 20617	Decrease in cholesterol levels	Al-Saleh et al. (2006)
Supplement containing *B. lactis*, *L. acidophilus*, and *L. rhamnosus*	Reduction of glucose levels in blood	Al-Salami et al. (2008)
Supplement containing *E. faecium* RM11 and *L. fermentum* RM28	Reduction in risk of colorectal cancer	Thirabunyanon et al. (2009)
Lacidofil probiotic (*L. rhamnosus* R0011 and *L. acidophilus* R0052)	Improvement of the irritable bowel symptoms, mental health, and cancer-related fatigue in colorectal cancer patients	Lee et al. (2013)
B. adolescentis SPM0212	Inhibition of the proliferation of human colon cancer cell and fecal enzymes	Kim et al. (2008)
Supplement containing *L. acidophilus* 4356	Decrease in cholesterol levels	Huang et al. (2010)
Supplement containing *L. casei* Shirota	Reduction of anxiety symptoms	Rao et al. (2009)
Supplement containing *L. rhamnosus GG*	Antidiabetic effect	Tabuchi et al. (2003)

Table 2 (continued)

Microorganism/product	Observed effect	References
Supplement containing *L. helveticus* R0052 and *B. longum* R0175	Mental well-being (decrease in levels of stress)	Messaoudi et al. (2011)
Supplement containing *L. plantarum* DSM 9843	Pain relief and reduction in flatulence (irritable bowel syndrome)	Nobaek et al. (2000)
Supplement containing *L. rhamnosus* GG, *L. rhamnosus* Lc705, *Propionibacterium freudenreichii* ssp. *shermanii* JS, and *B. animalis* subsp. *lactis* Bb-12	Stabilization of intestinal microbiota and reduction in symptoms of irritable bowel syndrome	Kajander et al. (2008)
Supplement containing *B. polyfermenticus* SCD	Modulation of physiological functions (lipid and antioxidant profiles in hypercholesterolemia)	Paik et al. (2005)
Supplement containing *B. pumilus* SE5	Modulation of intestinal microbiota	Sun et al. (2011)
Probiotic mix VSL#3 (*L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *S. salivarius* subsp. *thermophilus*)	Modulation of intestinal microbiota in patients with irritable bowel syndrome	Ng et al. (2013)

The consumption of probiotics has shown positive effects on different disorders such as diabetes, hypercholesterolemia, and high blood pressure. A study published by Al-Salami et al. (2008) showed that the administration of probiotics (*L. acidophilus*, *B. lactis*, and *L. rhamnosus*) reduced the glucose levels in the blood of diabetic rats, thus exerting a hypoglycemic effect. According to Tabuchi et al. (2003), a supplement containing *L. rhamnosus* GG was administered to rats and also presented an antidiabetic effect, which could be attributed to the prevention of the decrease in insulin secretion. When used as a supplement, *B. polyfermenticus* SCD showed significant health benefits by modulating the physiological functions, including the lipid and antioxidant profiles in hypercholesterolemic rats (Paik et al. 2005). Ataie-Jafari et al. (2009) demonstrated that the ingestion of two strains of probiotic bacteria (*L. acidophilus* and *B. lactis*) for 6 weeks was associated with a cholesterol-reducing effect in hypercholesterolemic individuals due to the inhibition of cholesterol absorption or its assimilation/capture by the bacteria cell membrane. Furthermore, the administration of a supplement containing *L. acidophilus* 4356 to rats for 4 weeks reduced the cholesterol levels (Huang et al. 2010). Sindhu and Khetarpaul (2003) reported that the consumption of a fermented cereal added with probiotics (*L. casei* NCDC-19 and *Saccharomyces boulardii*) for 42 days was capable of reducing the total cholesterol levels in mice. Finally, the ingestion of fermented milk containing probiotic *Lactobacillus helveticus* was shown to reduce the blood pressure of both hypertensive rats (Jauhiainen et al. 2005) and individuals (Seppo et al. 2003) and, therefore, could be useful in the treatment of the condition.

Allergic diseases

Atopic diseases are caused by exaggerated or nonequilibrated immunological responses to environmental and inoffensive antigens (allergens) (Sanders et al. 2007). Allergic diseases can be initiated and maintained by environmental factors associated with a change in the intestinal microbiota (Prakash et al. 2011). A study carried out by Wang et al. (2004) showed that the symptoms of patients suffering from allergic rhinitis were alleviated by the consumption of a fermented milk added with *Lactobacillus paracasei*-33, through changes in the composition of the gut microbiota. According to Kirjavainen et al. (2003), a supplement containing *L. rhamnosus* GG showed potential for the treatment of atopic eczema and allergy to cow's milk in children.

Mental diseases

Probiotic bacteria have an important role in bidirectional communication of the intestine-brain axis and can be used as therapeutic adjuncts in stress-related disorders, such as anxiety and depression. When administered to mice, *L. rhamnosus* JB-1 reduced anxiety and depression symptoms via modulation of the intestinal microbiota due to the bidirectional communication between the brain and the gut (Bravo et al. 2011). Similarly, Rao et al. (2009) reported a reduction of anxiety symptoms in patients who used for 2 months a supplement containing *L. casei* Shirota. Moreover, fermented milk added with *L. helveticus* IDCC3801 prevented and alleviated Alzheimer's disease and other memory dysfunctions in mice via reduction of accumulation of neurotoxic peptides, which are involved in the development of the disease (Yeon et al. 2010). Moreover, the administration of a supplement containing *L. helveticus* R0052 and *B. longum* R0175 promoted mental well-being to patients, including a decrease in stress levels, which was associated with the stress state of the individual (Messaoudi et al. 2011).

Periodontal diseases

Gingivitis is an inflammatory reaction caused by the accumulation of bacteria in the gingival gums. Once inflammation and degradation of the collagen increase, the process can lead to periodontitis (Yanine et al. 2013). Some studies have demonstrated the effectiveness of probiotics in the improvement of oral health. *Lactobacillus reuteri* was shown to be able to reduce gingivitis and bacterial plaque in subjects with moderate and severe gingivitis (Krasse et al. 2005). Probiotics lozenges containing *L. reuteri* were shown to be useful in the treatment of patients diagnosed with periodontitis (Teughels et al. 2013). According to Maekawa and Hajishengallis (2014), the topical treatment with *L. brevis* CD2 inhibited the periodontal inflammation in mice through modulatory effects on the periodontal microbiota. Bhardwaj and Bhardwaj (2012) hypothesized that probiotics may improve the signals/symptoms of the disease by controlling the growth of periodontal pathogens.

Bacterial infections

The susceptibility to enteric infections increases with the disrupture of the commensal microbiota equilibrium (Prakash et al. 2011). The consumption of a fermented milk containing *L. plantarum* and *L. casei* led to a decrease in both stool recovery and intestinal colonization rates of *Escherichia coli* O157:H7 in rats and, consequently, minimized the duration and severity of the infection (Mirzaei et al. 2012). According to VidyaLaxme et al. (2012), a food product containing ragi malt and probiotics *Bacillus subtilis* natto RG4365 and *Leuconostoc mesenteroides* inhibited the planktonic growth of *Vibrio cholerae* and affected its ability of biofilm formation and adherence to extracellular matrix proteins. The authors also observed increased amounts of beneficial fatty acids such as linoleic and linolenic acids and higher mineral contents (iron and zinc) when both microorganisms were added to the functional food product, in comparison to the amounts determined when *B. subtilis* natto RG4365 and *L. mesenteroides* were tested alone.

Colorectal cancer

Colorectal cancer is mainly a disease of developed countries with a Western culture, and adenocarcinoma is considered the most common type of the disease (Prakash et al. 2011). Studies have shown an association among the intestinal microbiota, colorectal cancer, and the administration of probiotics (Kim et al. 2008; Levy et al. 2014). *B. polyfermenticus* SCD showed anticarcinogenic effect in rats, which was explained by its strong adherent properties in the intestinal mucosa and also by the inhibition of the growth of human colon cancer cells (Lee et al. 2007). According to Thirabunyanon et al. (2009), in vitro tests demonstrated that *Enterococcus faecium* RM11 and *L. fermentum* RM28 were able to reduce the proliferation of colon cancer cells through the reduction of the viability and induction of the apoptosis of colon cancer cells due to an increase of the adherence of the probiotics to the intestinal epithelial cells. The administration of probiotic Lacidofil containing *L. rhamnosus* R0011 and *L. acidophilus* R0052, for 12 weeks, improved cancer-related fatigue in colorectal cancer patients (Lee et al. 2014). The authors correlated the positive outcome with the probiotics action mechanisms, which could have included the restoration of the gut flora and their anti-inflammatory properties.

Modulation of the immune system

Autoimmune diseases occur when the body's immune system attacks and destroys healthy tissues, as in the case of type 1 diabetes (Boerner and Sarvetnick 2011), celiac disease (de Sousa Moraes et al. 2014), inflammatory bowel diseases (Kostic et al. 2014), and allergic asthma (Bach 2002). Several studies have shown that probiotics can act by modulating the immune system. Capsules containing *B. coagulans* strains GBI-30 and 6086, administered to patients, led to an increase in the levels of immunological markers including cytokines (IL-6, IL-8, and IFN-γ) and CD3CD69 cells (Kimmel et al. 2010). According to Amit-Romach et al. (2010), the administration of *L. rhamnosus* GG affected the innate immunity in the intestine of colitis-induced rats through the increase of the mucin expression, which is important in the inhibition of the adherence of pathogenic bacteria in the gut. Furthermore, the probiotic treatment reduced the expression of proinflammatory cytokines, tumor necrosis factor-α, and IL-6 and, consequently, reduced the inflammatory response. According to Hougee et al. (2010), the treatment using *B. breve* M-16 V in allergic asthma-induced mice attenuated the allergic symptoms due to the reduction of the lung inflammation and the IgE and IgI levels. Moreover, a study carried out by Takeda et al. (2011) showed that heat-killed *L. plantarum* 06CC2 alleviated influenza symptoms in mice by an increase of natural killer cell activity associated with the enhancement of interferon-α and Th1 cytokine production. Furthermore, Medici et al. (2004) demonstrated that the consumption of cheese containing *B. bifidum*, *L. acidophilus*, and *L. paracasei* exerted an immune-modulating effect on mice intestines via interaction of the probiotics with the immune cells of the gut. Finally, Martinez et al. (2009) demonstrated that cell-free supernatants from probiotic strains *L. reuteri* RC-14 and *L. rhamnosus* GR-1 were able to upregulate the secretion of cytokines (IL-8 and IP-10) by vaginal epithelial cells infected with *Candida albicans*. The authors concluded that this mechanism could possibly play an important role to help clear out vulvovaginal candidiasis in vivo.

Conclusions

Until now, although traditional and molecular methods are available for the characterization of the intestinal microbiota, a full understanding of its composition and diversity and how changes in this microecosystem cause or are associated with the development of diseases seems to be beyond our grasp. Thus, the performance of more in vitro and in vivo studies that analyze the diversity, function, and action mechanisms of GIT microorganisms and also elucidate how probiotics can positively affect/interact with the intestinal microbiota is essential for the development of new strategies to prevent/manage several relevant pathologic conditions.

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