# Next-Generation Probiotics Their Molecular Taxonomy and Health Benefits


**Shams Tabrez Khan and Abdul Malik**

**Abstract** The concept of probiotics although perceived as new is more than a century old. Since the early studies of the Elie Metchnikoff in 1903, a number of commercial products containing probiotics are in the market. The recent success of converting probiotic products into commercial reality was achieved by the scientists like Minoru Shirota and Kellog. Minoru Shirota is a Japanese scientist who successfully demonstrated the health benefits of probiotics and commercialized the globally known probiotic drink Yakult. This renewed interest in probiotics is spurred by the recent advances made in understanding the human microbiome and its role in human health. The link between the gut microbiome and human health is becoming increasingly clear and is well described. Nevertheless, the gut microbiome is continuously influenced by a number of factors like diet, lifestyle and consumption of antibiotics. A healthy gut microbiome can be retained and maintained by using various probiotics. Moreover, the probiotic microorganisms are no more limited to a few conventionally used bacteria and are being currently represented by more phylogenetically diverse microorganisms than previously thought. These probiotic microorganisms include conventionally used Lactic acid bacteria, like *Lactobacillus* and recently identified probiotic bacteria like *Akkermansia muciniphila*, *Bifidobacterium infantis*, *Bacteroides fragilis*, *Clostridium butyricum*, *Faecalibacterium prausnitzii* and *Streptococcus thermophiles* etc. Many of these probiotic strains have a shared mechanism of action, while strain specific, species-specific or genus-specific probiotic effects have also been documented. Probiotics are administered as live cultures or as spores, directly or through fermented dairy products, food, and drinks. Probiotics based therapies like fecal microbiota transplant are also being used successfully for treating medical conditions and diseases like diarrhea, constipation, vaginitis, necrotizing enterocolitis, inflammatory bowel disease, *Clostridium difficile* infection, and others. Reports showing a clear role of probiotics in immunomodulation, prevention of cardiovascular diseases and even cancer are also emerging. Yet, a number of microorganisms in the gut remain uncultured and many candidate probiotic

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microorganisms remain poorly identified, requiring correct identification and a rigorous evaluation as probiotics. Probiotics may be a century old but require fresh attention keeping in view the recent advances made in understanding the gut microbiome and the role of these microorganisms in human health.

**Keywords** Probiotics · Health benefits · Molecular taxonomy · Microorganisms

## Introduction

The name Probiotics originated from the Latin word "*pro*" meaning "for" and the Greek "*βιοσ*" meaning "Life". Werner Kollath (1953) used the term for active substances that are essential for healthy development of life (Gasbarrini et al. 2016). History of the use of probiotics is as old as 10,000 years, but the more recent work of the Russian scientist Elie Metchnikoff (1900s) is considered as pioneer work on probiotics (Ozen and Dinleyici 2015). Though Louis Pasteur did the pioneering work on fermentation but the effect of fermented products on human health was studied by Elie Metchnikoff. He associated the long-life expectancy of Bulgarian people with the consumption of yogurt containing the bacterium referred to as Bulgarian Bacillus (Mackowiak 2013). His conclusions that the intestinal microbes depend on the food, and the type of food may help in modifying the flora in our bodies and to replace the harmful microbes by useful microbes, clearly laid the foundation of the probiotic concept (Burki 2018). Metchnikoff actually used the term "Orthobiosis" in his publications entitled, "The nature of man: Studies in Optimistic Philosophy (1903)" and "The Prolongation of Life: Optimistic Studies (1907)" for natural ways for delaying senility and for health (Podolsky 2012). Although a number of products containing probiotic bacteria were in use traditionally, the commercialization of Yakult in 1930 is undoubtedly a global success story of probiotic commercialization.

Since then the research on probiotics was largely ignored till the revolutionization of DNA sequencing techniques in the late 1990s. These techniques greatly improved our understanding of human microbiome and its role in health and disease (Cho and Blaser 2012). The role of microorganisms in many diseases is becoming increasingly clear (Fong 2014; Neish 2009; Macpherson and Harris 2004). Even the problems of obesity, some cancers, and metabolic syndrome are being associated with the unhealthy microbiome (Vrieze et al. 2012; Boulangé et al. 2016; Chang and Parsonnet 2010). Therefore, from the current understanding of the human microbiome and their role in controlling various diseases new evidences have been gathered in support of the theories of Metchnikoff. Furthermore, many more new microorganisms are being identified as probiotic, and the mechanism of their probiotic activities are now better understood. These probiotics are often referred to as next-generation probiotics and are discussed in this chapter.

## Prebiotics, Postbiotics/Parabiotics, and Synbiotics

Since, many terms including prebiotics, postbiotics, and synbiotics are in use it is necessary to discuss these terms briefly before a detailed discussion on probiotics. Prebiotics can be defined as nutrients that favor the growth of probiotic bacteria in the gut (Delgado-Fernández et al. 2019). Some of the well-known prebiotics include fructan and nonfructan oligosaccharides (Anadón et al. 2016). While, the postbiotics/parabiotics can be defined as functional foods that contain probiotic effector molecules in the form of nonviable probiotic organisms, or cell lysates resulting in the required health benefits of probiotics. Generally, these postbiotics include molecules like acetaldehydes, bacteriocins, organic acids, and hydrogen peroxide. Recent studies suggest that effector molecules present in the diet even in the absence of producer probiotic organism show the probiotic activity. For example, it has been demonstrated that muramyl dipeptide-based postbiotics curtails liver insulin resistance and fat inflammation via NOD2 (Cavallari et al. 2017). Synbiotics are functional foods or formulations that contain both probiotics and prebiotics. Since, synbiotics are a combination of both probiotics and prebiotics, these formulations enhance the establishment and selection of probiotic bacteria in the gut (Mohanty et al. 2018). An overview of the four terms is shown in Fig. 1.

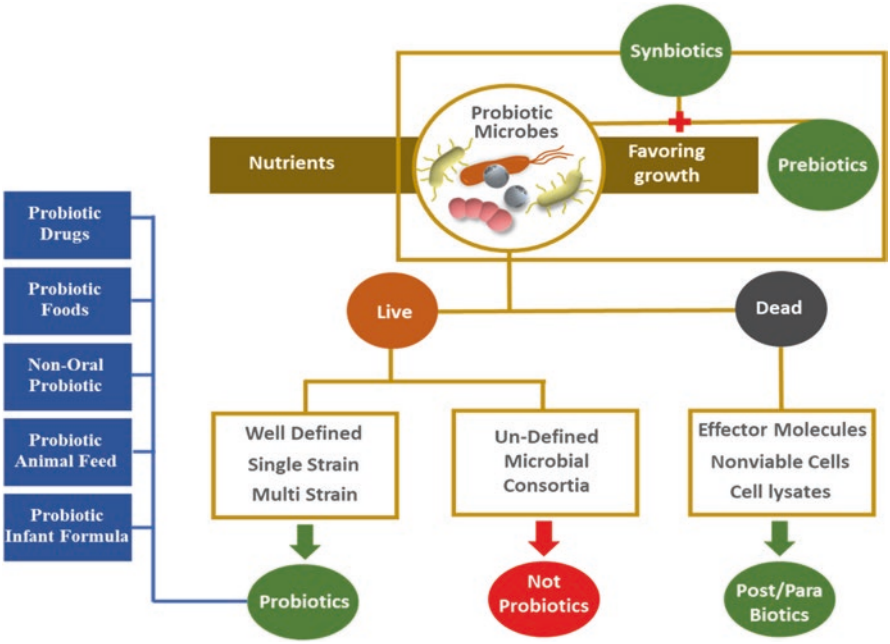


**Fig. 1** The concept of probiotics, prebiotics, post/parabiotics and synbiotics

## Definition of Next-Generation Probiotics

Though there is no legal definition of Probiotics for regulatory purposes in the United States. Various definitions for probiotics have been proposed earlier (Sanders 2008). For example, Fuller described probiotics as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" (Fuller 1989). Havenaar and Huis In't Veld defined probiotics as follows "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora" (Havenaar and Huis In't Veld 1992). A more recent definition of probiotics is "live microorganisms, which when consumed in adequate amounts, confer a health effect on the host" (Guarner and Schaafsma 1998). This definition has been slightly grammatically modified by the International Scientific Association on Probiotics and Prebiotics (ISAPP). ISAPP has redefined probiotics as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*" (Hill et al. 2014). This definition is widely accepted, which may also change based on the new knowledge. However, as of now, the next generation probiotics can be defined as above. Other terms are also used for probiotics such as live therapeutic products.

## Guidelines for the Use of Term Probiotics

Widespread commercialization of probiotics requires clear guidelines for identifying probiotic strains, their safety evaluation, and regulation. This has also renewed the interest of Scientific community in probiotics and as of 11 Jan 2019, there were more than 20,000 and 30,000 documents in PubMed and ScienceDirect on probiotics, respectively. Many commercial products claim unsubstantiated health benefits of the probiotic products. Furthermore, although the use of undefined gut microbial communities such as fecal microbiota transplants as probiotics is also becoming increasingly acceptable. But the use of such undefined microbial communities has its own potential risks. These developments should be properly regulated based on controlled studies. World health organization and Food and Agriculture Organization (FAO) organized expert meetings in 2001 and 2002 to issue a consensus statement for using the term probiotics and other guidelines related to probiotics. It was agreed in the meeting that the term probiotic should only be used for microbial species exhibiting clear health benefits in properly controlled studies. Commercial products should only use the term "contains probiotics" and any other claim regarding the health benefit should be substantiated. The use of live microbial species without any known health benefit should be discouraged and the term probiotic should not be used for such microbial species. Similarly, undefined microbial communities should not be defined as Probiotics. While, defined microbial consortia of human origin with known health benefits and safety can be defined as Probiotics (Table 1) (Hill et al. 2014).

**Table 1** Some of widely used probiotic strains

| Genus | Species and strain | Reference |
|---|---|---|
| *Lactobacillus* | *L. rhamnosus* GG, *L. acidophilus* NCFM, *L. casei* Shirota, *L. reuteri* MM53, *L. rhamnosus* GR-1, *L. fermentum* RC-14 | Reid (1999) |
| *Bifidobacterium* | *B. lactis* HN019, *B. longum* CECT 7210, *B. catenulatum*, *B. breve* Yakult, *Bifidobacterium bifidum* NCFB 1454, *B. animalis* | Vlasova et al. (2016) |
| *Bacteroides* | *B. uniformis* CECT 7771, *B. fragilis* | El Hage et al. (2017) |
| *Bacillus* | *B. coagulans* 15B, *B. subtilis* CU1, *B. licheniformis* CH200 | Elshaghabee et al. (2017) |
| *Streptococcus* | *S. thermophilus* FP4 | Jäger et al. (2016) |
| *Clostridium* | *C. butyricum* MIYAIRI 588 | SEKI et al. (2003) |
| *Enterococcus* | *E. faecium* K77D | Hanchi et al. (2018) |
| *Akkermansia* | *A. muciniphila* | Cani and de Vos (2017) |
| *Faecalibacterium* | *Faecalibacterium prausnitzii* | Martín et al. (2017) |

## Phylogenetic Diversity of Next-Generation Probiotics

Understanding the taxonomy of probiotic bacteria is key to their commercialization, since one strain of the same species may exhibit probiotic properties while other strain may not (Campana et al. 2017). It is recommended that the strains should be identified using International Code of Nomenclature and should be deposited in an Internationally recognized microbial culture collection (Morelli and Capurso 2012). Species-level identification should be based on DNA-DNA hybridization and 16S rRNA gene sequences. While, the strain level identification should be based on pulse field gel electrophoresis and randomly amplified polymorphic DNA or RAPD (Hill et al. 2014; Morelli and Capurso 2012). With the discovery of improved sequencing technologies more sophisticated approaches like whole genome sequencing, average nucleotide identity and multilocus sequence analysis is also being used to better understand the taxonomy of the closely related probiotic strains (Diancourt et al. 2007; Huang et al. 2018). The whole genome of hundreds of the probiotic strains including the members of the genus *Bacillus*, *Bifidobacterium*, *E. coli*, and *Lactobacillus* has been published (Siezen and Wilson 2010; Lukjancenko et al. 2012; Kang et al. 2017). However, even with the genome sequences of many strains available it remains difficult to have clear guidelines to delineate different strains as the genetic diversity of strains within a species varies greatly (Truong et al. 2017).

One of the oldest and well documented probiotic bacteria identified by Metchnikoff is *Lactobacillus bulgaricus* referred by him as Bulgarian Bacillus. Since then various species of *Lactobacillus* have been traditionally used as probiotics (Gasbarrini et al. 2016). Currently, the probiotics are not only limited to *Lactobacillus* and a number of different bacteria have been identified as probiotic bacteria. Which also necessitates a clear definition of probiotics, better regulatory guidelines and distinct guidelines for identifying the probiotics. For oral and dietary formulations bacteria belonging to different genera are being used in various commercial products. Some of the most widely used next-generation probiotics bacteria belong to the species of *Akkermansia*, *Bacteroides*, *Bifidobacterium*, *Clostridium*, and *Faecalibacterium* (Hill et al. 2014; Saarela 2019). It is to be noted that most of the genera listed above are Gram-positive bacteria but contrary to popular belief probiotic bacteria may also belong to Gram-negative bacteria such as *Akkermansia* and *Escherichia coli* Nissle 1917 (EcN). The latter being a well-studied probiotic bacterium (Behnsen et al. 2011). Other studies have claimed that the members of the genera *Pediococcus*, *Streptococcus*, *Propionibacterium*, and *Saccharomyces* also exhibit probiotic properties. Studies on gut microbiome have revealed that some genera though not the part of conventional probiotic food are associated with a healthy and robust gut microbiome. Strains of *Akkermansia muciniphila*, *Eubacterium hallii*, *Faecalibacterium prausnitzii*, and *Roseburia* spp. are not only associated with robust gut microbiome but also demonstrate clear health benefits in studies on animals. The suitability of these probiotics for their use in food is yet to be evaluated. Various species and strains of these genera identified as probiotics are shown in Fig. 2, which shows a phylogenetic tree of these bacteria based on 16S rRNA gene sequences.

Two of the most widely used genera as probiotics are *Lactobacillus* and *Bifidobacterium* belonging to phylum Firmicutes and Actinobacteria, respectively. The genus *Bifidobacterium* was originally described by Orla Jensen in 1924 and currently contains seventy species and ten subspecies (Orla-Jensen 1924). The current status of the Genus *Bifidobacterium* based on the genome sequences of 233 strains including the genomes of type strains is discussed in details by Lugli and colleagues (Lugli et al. 2018). Some of the species of Genus Bifidobacterium that are commonly used as probiotic include *B. adolescentis*, *B. animalis*, *B. bifidum*, *B. breve* and *B. longum*. The genus *Lactobacillus*, *however*, is a traditionally known probiotic bacteria which was officially published by Beijerinck in 1901. The genus currently contains 237 species and 29 subspecies. It is interesting to note that close to 200 species from the genus are used as probiotics (Salvetti et al. 2018). Some of the notable species of Lactobacillus used as probiotic include *L. acidophilus*, *L. casei*, *L. fermentum*, *L. gasseri*, *L. johnsonii*, *L. paracasei*, *L. plantarum*, *L. rhamnosus*, and *L. salivarius*. Salvetti and colleagues have discussed the recent advances in the taxonomy of the genus *Lactobacillus* and have suggested the presence of ten subclades within the genus (Salvetti et al. 2018). *Bacteroides* spp. are also used as probiotics and are found in high numbers in gut. *Bacteroides thetaiotaomicron*, *Bacteroides fragilis* and other species of Bacteroides can efficiently metabolise complex polysaccharides, produce short chain fatty acids and are known to modulate host immune

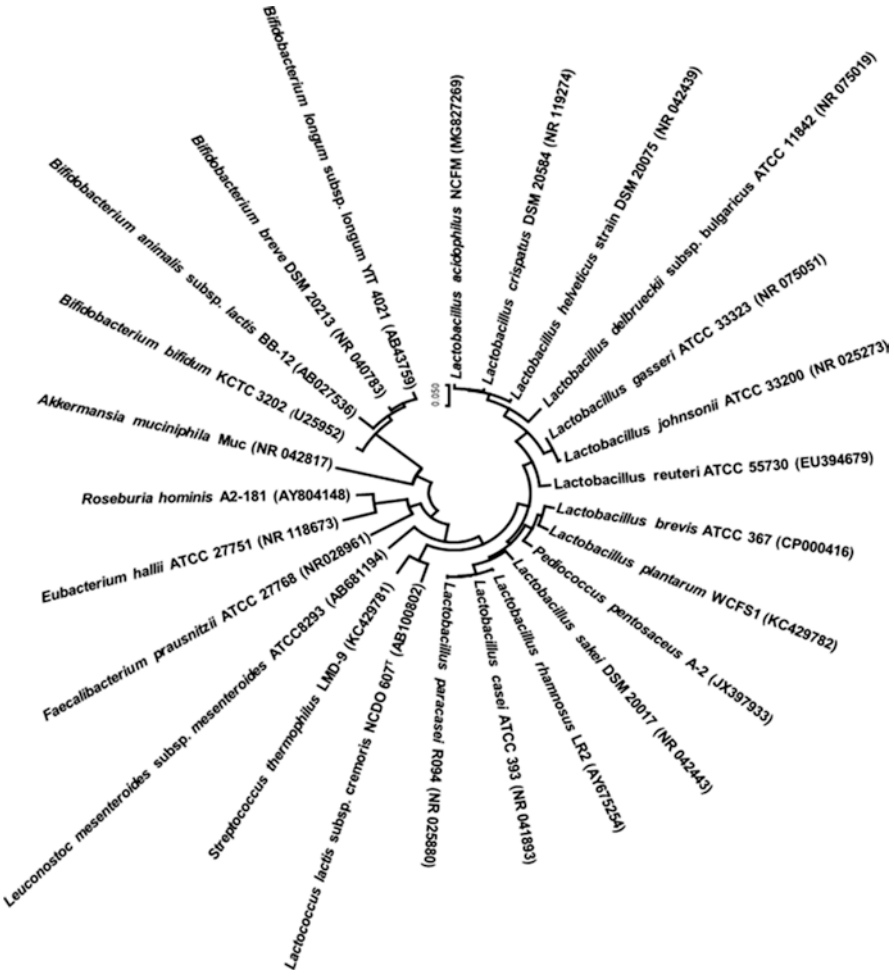


**Fig. 2** Phylogenetic tree based on the 16S rRNA gene sequence showing the phylogenetic positions of some commonly used probiotic bacteria

system. Another potential probiotic that has been studied in details is *Clostridium butyricum* MIYAIRI 588. This strain is already available commercially as supplements. The role of the strain to provide protection against the infections of *E. coli* O157:H7 in gnotobiotic mice has been documented (Hayashi et al. 2013; Murayama et al. 1995). The use of *Clostridium butyricum* MIYAIRI 588 for animal feed is already authorized by the European Union (Saarela 2019). *Faecalibacterium prausnitzii* is a Gram-negative butyrate producing bacterium commonly found in the gut. In addition to the production of butyrate the bacteria also have immunomodulatory effect on the host. The ability of the bacterium to contain diarrhea in dairy calves has also been documented (Foditsch et al. 2016).

## Next-Generation Probiotics: How to Select

Since the market for the probiotics is growing rapidly and a number of new probiotic candidates are being isolated and identified for their intended use by humans. A candidate strain must be thoroughly screened for a number of characteristics before their use as probiotics is finally approved, the first and the most important being the correct taxonomic identification of an organism to strain level. Identification of the health benefits associated with a candidate organism especially the core health benefits associated with probiotics (Hill et al. 2014). Furthermore, it is important to ensure that a candidate strain must not have properties that in any way may harm the consumer host such as infectivity and pathogenicity. A candidate strain must also meet the criteria described by regulatory authorities and should also be suitable for industrial production. For example, it should have adequate growth rates etc. This makes selection of probiotic microorganisms a systematic approach wherein a step by step screening of a candidate strain is involved (de Melo Pereira et al. 2018). Some of the important traits are discussed below and a brief outline is given in Fig. 3.

### *Correct Taxonomic Identification*

Correct identification of a candidate strain is key to its success as it has been observed that many strains have strain-specific health benefits. It is recommended by the regulatory authorities like FAO that a combination of phenotypic and modern genotypic techniques should be employed for the identification of the organism at genus, species and strain levels. The use of modern techniques like 16S rRNA gene

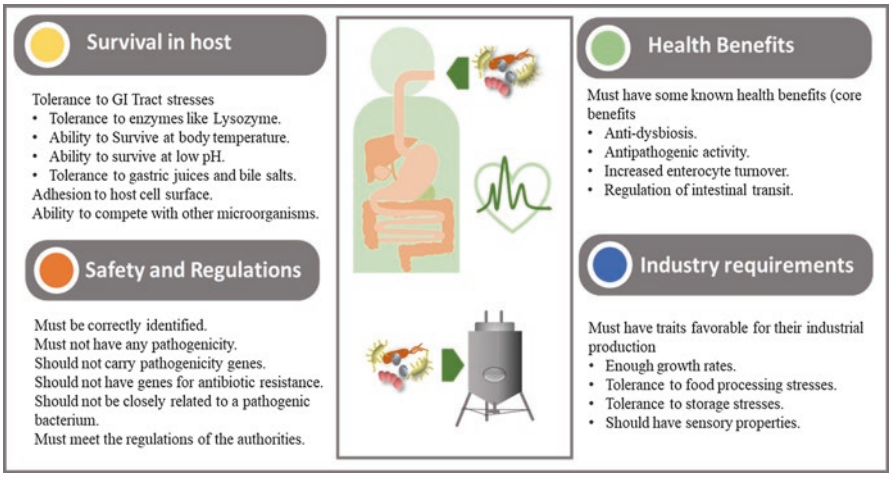


**Fig. 3** A summary of parameters used for the selection of a candidate probiotic strains

sequence analysis, Fatty acid analysis, Pulse field gel electrophoresis, and even the use of whole genome sequence is also recommended. Furthermore, the old names should be replaced with new names if required. WHO also recommends that the probiotic strains should also be deposited in internationally recognized microbial culture collections (Huys et al. 2013).

## *Tolerance to GI Tract Related Stress*

It is important for a candidate probiotic strain that it should tolerate the stresses posed by the human body Fig. 3. Once a candidate strain is given orally it should be resistant or tolerant to enzymes present in the oral cavity especially lysozyme. As most of the Gram-positive bacteria are sensitive to lysozyme, while some Lactobacillus bacteria (LAB) are resistant enough to be the part of human oral microbiome (Kõll et al. 2008). During the further passage of bacteria into the GI tract, the bacterium will be exposed to gastric juices and pepsin in the stomach and to the bile juices secreted by the liver. Therefore, a candidate probiotic strain should be tolerant to bile juices. It has been demonstrated that *Lactobacillus*, *Bifidobacteria* and many other probiotic bacteria contain Bile Salt Hydrolase (BSH) activity (Begley et al. 2006). Although the tolerance or resistance to various stresses varies with the probiotic strain. But it is recommended that the probiotic strains should be tolerant to a pH range of 2–5 and to a bile salt concentration range of 0.3–2% (Ogunremi et al. 2015). Mechanisms used by the probiotic bacteria to tolerate the GI tract stresses are reviewed in details by Bustos and colleagues (Bustos et al. 2018).

## *Potential to Colonize and Adhere to GI Tract*

Another important property of a candidate probiotic bacterium is their ability to adhere to GI tract and to consequently colonize the GI tract. Various studies have demonstrated the ability of probiotic bacteria in-vitro to adhere to the surfaces coated with intestinal mucin or to GI tract cell lines such as HT29 (Nishiyama et al. 2016; Turpin et al. 2012). The adhesion of a candidate probiotic strain depends on the surface properties of the host epithelial cells and the biochemical composition of the probiotic strain's cell surface. The extracellular material secreted by bacteria is also known to influence the adhesion significantly (Boonaert and Rouxhet 2000; do Carmo et al. 2018a). Auto aggregation of probiotic bacteria helps the bacterium to achieve a high cell density which consequently helps the bacterium to adhere to the intestinal surfaces. The hydrophobicity of Bacterial cell surface is another property that affects its binding to epithelial cells. In an interesting study, about 163 strains of *Lactobacillaceace* were isolated and were screened for the presence of 14 genes potentially involved in the binding. It was observed that some of these genes (*ef-Tu*, *gap*, *groEl*, and *srtA*) were housekeeping genes and were therefore present in all the

strains (Turpin et al. 2012). While, other genes (*apf*, *cnb*, *fpbA*, *mapA*, *mub1*, and *mub2*) were present only in 86–100% LAB bacteria tested. This study provided the genetic evidence for the binding properties of the probiotic bacteria.

## *Health Benefits/Activity Against Pathogenic Bacteria*

Probiotic candidates must have some distinct health benefits one of the most important being the antimicrobial activity against pathogenic bacteria. The antimicrobial activity of these probiotic bacteria maybe due to the chemical exclusion or through competitive exclusion. Chemical exclusion refers to the production of antimicrobial compounds like bacteriocins, enzymes, hydrogen peroxide and organic acids (Neal-McKinney et al. 2012; Dobson et al. 2012; Cotter et al. 2012). While, the competitive exclusion refers to antagonism through competition for nutrients and space for attachment (Lebeer et al. 2018; Callaway et al. 2008). There are many other health benefits associated with probiotic microorganism discussed below in details. For the screening of these traits, different assays are used (Papadimitriou et al. 2015).

## *Safety Assessment*

Although, the probiotics are generally recognized as safe (GRAS) but there are many reasons that make it important to assess the safety of the probiotics. One is the ever-increasing list of microorganisms that are being identified as probiotics. The presence of antibiotic resistance genes and the possible presence of genes for pathogenicity (Zheng et al. 2017; Kochan et al. 2011; Doron and Snydman 2015). Therefore, regulatory authorities like USFDA, WHO, EFSA of European Commission and NHPR of Canada are making new regulations for the safety assessment of probiotics. These new regulations require information on history of isolation, correct taxonomic identification of the candidate strain, and absence of harmful traits such as infectivity, virulence, toxicity and the presence of transferable antibiotic resistance genes (Sanders 2008; Venugopalan et al. 2010; Wright 2005).

## *Strain Stability, Viability, and Commercial Production Related Properties*

A number of other properties make a candidate strain suitable for its industrial production such as the ability to survive in the food, the ability to survive during storage without losing the viability and the ability to grow quickly during the fermentation to reach a desired optimal population.

## Source of Probiotic Isolation

Probiotic microorganisms can be isolated from a number of sources. Since probiotics have been used traditionally in food such as dairy products and fermented foods globally. These products are good source of probiotics. Dairy products include milk from different dairy animals, yogurt, cheese, and fermented milk etc. Human milk has also been used for the isolation of probiotic microorganism (de Melo Pereira et al. 2018). While other probiotic based products include fermented meat, fish, pickles, cereals, vegetables, miso, tempeh and others (Rezac et al. 2018). These products can also be used for the isolation of probiotic bacteria. Bacteria from the gut of healthy individuals are also good source of probiotic bacteria. Lactic acid bacteria (LAB) have been isolated from feces samples of children and adults for their potential use as probiotics (Rubio et al. 2014). One of the well-known probiotic *Escherichia coli* Nissle 1917 was isolated from the feces of a soldier (Behnsen et al. 2011). A detailed list of various sources of probiotics is given in the review of de Melo Pereira (de Melo Pereira et al. 2018).

## The Health Benefits and Mechanism of Next-Generation Probiotics

Plenty of literature is available demonstrating various health benefits of probiotic microorganisms (Behnsen et al. 2011). Probiotic microorganisms are not only known to help in the maintenance of healthy gut microbiome and treating gastrointestinal problems but are also known to treat and provide protection against many other microbial and non-microbial diseases including obesity and cancer (George Kerry et al. 2018; Song et al. 2018). Although the health benefits associated with various probiotic strains vary greatly, majority of the strains share some core health benefits. For example, majority of the probiotic bacteria produce β-galactosidase which helps in the digestion of lactose. The two most common benefits associated with probiotics are the maintenance of a healthy digestive tract and a strong immune system (Hill et al. 2014). The mechanisms through which these microorganisms exhibit probiotic activities have also been categorized into three classes. The first category which is widespread and is found in most of the probiotic microorganisms include resistance to colonization, production of short chain fatty acids, normalization of perturbed gut microbiota, increased turnover of enterocytes, competitive exclusion of pathogens and regulation of intestinal transit Fig. 4 (Hill et al. 2014). The second group of mechanisms that are less commonly found and are associated at species level include the production of vitamins, enzymatic activity, neutralization of carcinogens, bile salt metabolism and direct antagonism. While the third category of mechanisms are rare are found only in a few strains or they are strain-specific effects. These mechanisms include neurological effects, immunological effects, endocrinological effect and the production of specific bioactive compounds

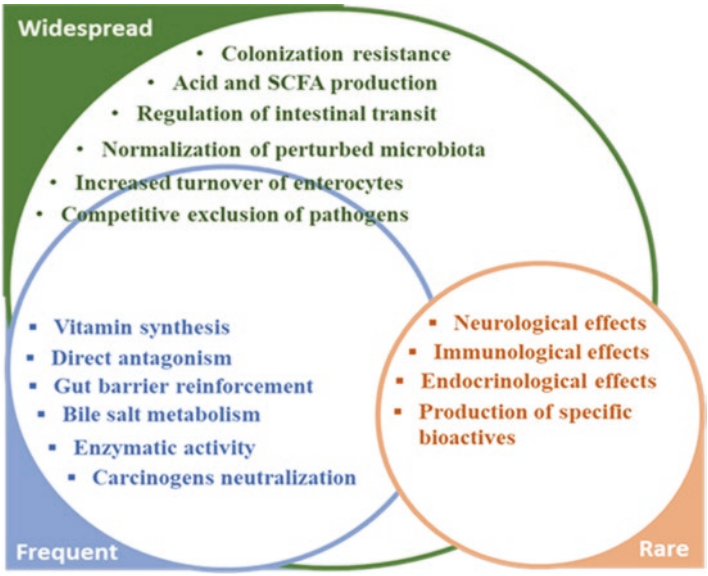


**Fig. 4** Categorization of different health benefits associated with different probiotic strains according to the frequency of their prevalence in different probiotic strains

(Hill et al. 2014). The main health benefits of probiotics discussed below, therefore, are the modulation of activity and composition of host microbiota, enhancement of epithelial barrier function, modulation of the immune system, modulation of systemic metabolic response and modulation of central nervous system signaling (Lebeer et al. 2018). For the delivery of the proper health benefits, it is also required that the strains should be present in enough numbers. It is required by Health Canada that the viable population should be in the range of $1 \times 10^9$ cells per serving (Hill et al. 2014). Regulations requiring similar viable count per serving are also in place in Italy. Some of the mechanisms through which probiotic bacteria exhibit their activity are shown in Figs. 4 and 5 and are listed in Table 2.

## *Role of Probiotics in the Treatment of Gastrointestinal Disorders*

One of the most important health benefits of probiotics is the maintenance of a healthy digestive tract microbiome. The human gut hosts around $10^{13}$–$10^{14}$ phylogenetically diverse microorganisms which are often perturbed by various factors such as food and the use of antibiotics (Kau et al. 2011; Gill et al. 2006). The microbiome present in the gut is mainly subdivided as the core, transient and variable microbiome (Derrien and van Hylckama Vlieg 2015). As the names suggest the core micro-

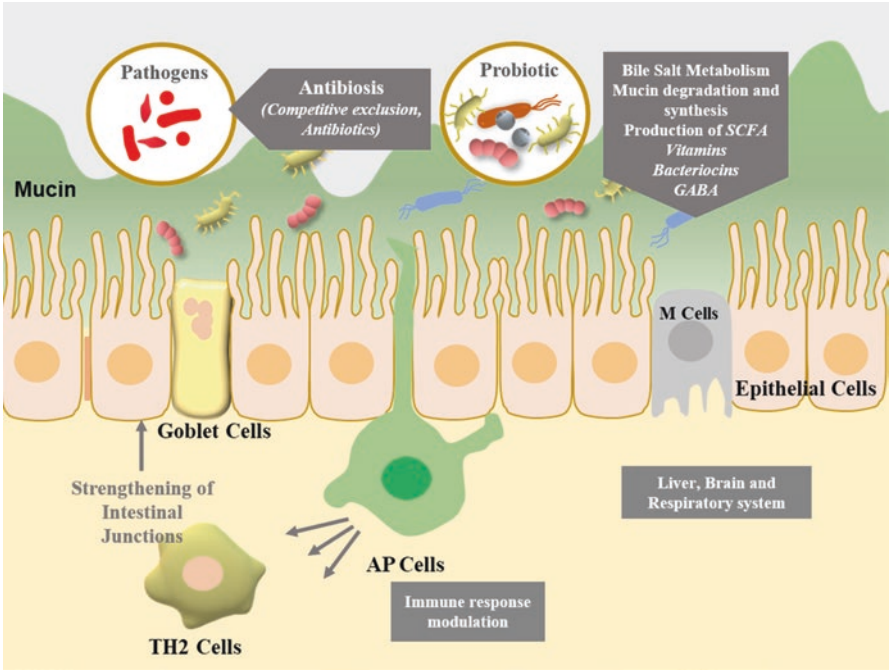


**Fig. 5** A schematic presentation of various mechanisms through which the bacteria exhibit their probiotic activity

**Table 2** Health benefits associated with some of the probiotic strains

| Bacterial strain | Health benefit | Reference |
|---|---|---|
| *Akkermansia muciniphila* | Ameliorates HFD-induced obesity and insulin resistance | Plovier et al. (2016), Zhou (2017) |
| Clostridia (clusters IV and XIVa) | Increased intestinal barrier function | Kelly et al. (2015), Kelly et al. (2005) |
| *B. fragilis* | Modulation of host metabolism | Chimerel et al. (2014), van Baarlen et al. (2013) |
| *Lactobacillus* spp. | Immunomodulation, Maintenance of mucosal homeostasis and intestinal barrier function | van Baarlen et al. (2013) |
| *Bifidobacterium* spp. | Reduced adiposity | Segovia et al. (2017) |
| *F. prausnitzii* | Improved insulin sensitivity | Villanueva et al. (2015) |
| *L. acidophilus* La5, *B. lactis* Bb12 | Inhibitory effect against *Helicobacter pylori* | Iannitti and Palmieri (2010) |
| *L. plantarum*, *L. reuteri* | treatment of IBS | Iannitti and Palmieri (2010) |
| *Bifidobacterium animalis* subsp. *lactis* CNCM I-2494 | Increased no of species producing butyrate, decrease the infection of *Bilophila* | Amoretti et al. (2002) |
| *L. reuteri* NCIMB 30242 | Increased Firmicutes/Bacteroidetes ratio | Martoni et al. (2015) |
| *Bacteroides fragilis* | Anti-inflammatory | Dasgupta et al. (2014) |

biome is made up of microorganisms that have been found in the human gut across the globe. The variable gut microbiome is made up of microbial species that are found in some humans but are absent from the gut of other humans. This variable microbiome doesn't change with diet perturbation etc. While the transient microbiome is the microbiome which is influenced by various factors like diet. The composition of the microbiome also changes with the part of the intestine (Derrien and van Hylckama Vlieg 2015) and age. The age influences the gut microbiome mainly due to the change in the type of diet and the change in immune system fortitude (Durack and Lynch 2019). Gut microbiome has been extensively studied and the relation with various diseases has also been reviewed extensively. Probiotic bacteria are now well known to effectively treat various intestinal ailments such as Inflammatory bowel disease (IBD), Diarrhea, Crohn's disease, Colitis, liver conditions and other intestinal disorders (Parker et al. 2018; Bermudez-Brito et al. 2012).

The two most commonly treated intestinal ailments using probiotics are Diarrhea and necrotizing enterocolitis (Kleerebezem et al. 2019). Various types of Diarrhea have been treated using probiotics including antibiotic-associated diarrhea (AAD), acute infectious diarrhea, and *Clostridium difficile*-associated diarrhea (CAD). The mechanisms used by probiotics to fight diarrhea include (a) competitive exclusion of pathogen causing diarrhea (b) Production of acids (like lactic acid and short chain fatty acids) consequently lowering luminal pH (c) production of bacteriocins, (d) promotion of mucus production enhancing epithelial barrier (Isolauri 2003) (e) production of β-galactosidase to help lactose digestion and (f) promoting the production of antimicrobial proteins cathelicidins and defensins by gut epithelium (Schlee et al. 2008). The strains of probiotic bacteria that are known to treat the diarrhea and details of the mechanisms have been summarized in reviews published earlier (do Carmo et al. 2018b). Enterocolitis especially the necrotizing enterocolitis is an inflammatory bowel disease (IBD), mainly affecting premature infants. The disease may result in bloody diarrhea, bloating and sensitive abdomen. Probiotic bacteria are known to treat the disease, *Bifidobacterium* is found to be more effective than any other probiotic bacteria (Kleerebezem et al. 2019). The mechanism involves the ability of the bacterium to utilize human milk oligosaccharides. While *Bifidobacterium longum* subsp. *infantis* is known to downregulate TLR4 by secreting a small glycan consequently preventing epithelial inflammatory response (Meng et al. 2016). Another probiotic bacterium *Lactobacillus rhamnosus* GG suppresses the inflammatory response in the intestine and the expression of TLR3 and TLR4. Crohn's disease (CD) is another prominent IBD which has been associated with the increase in the population of Enterobacteriaceae. While the anti-inflammatory role of certain symbiotic taxa like *Faecalibacterium prausnitzii* has been demonstrated (Sokol et al. 2017). *Lactobacillus lactis* which expressed the anti-inflammatory molecule from *F. prausnitzii* reduced the intestinal inflammation in mice (Quévrain et al. 2016).

## *Probiotics for the Prevention of Obesity and Type 2 Diabetes Mellitus (T2DM)*

Obesity is one of the major global health problems and a disturbed gut microbiome is one of the important reasons of obesity (Thaiss 2018). Even the microbiome distinctly associated with obesity has been identified (Turnbaugh et al. 2006). This obesity-related microbiome is often characterized by a reduction in the population of *Bacteroides* spp. and is capable of degrading the flavonoids in the diet (Durack and Lynch 2019). Which results in harnessing of much higher energy from the diet (Thaiss 2018). Another mucin-degrading gut bacteria is *Akkermansia muciniphila* which is often referred to as anti-obesity bacterium (Dao et al. 2016). This bacterium is also known to prevent other diseases such as IBD, hypertension and liver diseases (Cani and de Vos 2017). It has been documented that the polyphenols from the plants in the diet enrich *A. muciniphila*. The outer membrane protein of the bacterium interacts with TLR2 of the host helping in the restoration of gut barrier function (Anhê et al. 2015; Plovier et al. 2016). Furthermore, *A. muciniphila* can enhance the population of *Bacteroides* spp. which is the part of healthy gut microbiome (Gibson and Roberfroid 1995). The gut microbiome undergoes a transient change in its composition and function with the time in a day and these changes are referred to as circadian oscillations. It is reported that in obese persons these circadian oscillations are disturbed. In addition to the microbial interventions, an inulin-type fructans based diet can also improve metabolic disorders associated with obesity, such as a decreased fat mass, insulin resistance, lower liver steatosis and fortification of the gut barrier (Cani and de Vos 2017).

## *The Role of Probiotics in Immunity*

A number of probiotic microorganisms are known to modulate adaptive and innate immunity through well-understood mechanisms (Yan and Polk 2011). These mechanisms involve the change in gene expression, protein synthesis, and modulation of signaling pathways in immune and intestinal epithelial cells (Yan and Polk 2011). Probiotic microorganisms modulate the functions of dendritic cells, macrophages, and T and B lymphocytes. It has been found that bacteria like *Bifidobacterium adolescentis* modulates the GI T helper 17 cells (Ivanov et al. 2009). These bacteria play a key role in the maintenance of barrier function and provide protection against pathogenic microbes through the production of antimicrobial peptides (Pandiyan et al., 2011; Wang et al., 2014). Short chain fatty acids produced by *Clostridium* species induce +CD4+Foxp3+ T reg cells regulate T cell-mediated host immune responses. Surface polysaccharide of *Bacteroides fragilis* has been shown to bind to Toll-like receptor 2 on dendritic cells (DCs), which subsequently induces the production of the anti-inflammatory cytokine IL-10 by T reg cells and promotes immune tolerance (Dasgupta et al. 2014). It also has been observed that variation in

microbiome lipopolysaccharide (LPS) immunogenicity results in autoimmunity in humans (Vatanen et al. 2016). In this study it was found that the LPS from genus *Bacteroides dorei* inhibits the immunostimulatory activity of *Escherichia coli* LPS.

## *Treatment of Atopic asthma*

Asthma is a serious disease affecting 300 million individuals worldwide. It is an inflammatory disease of the airway, leading to hyper-production of mucus in the airway hyperresponsiveness and obstruction of the airway (Kudo et al. 2013). Asthma is a well-known T helper cell's (Th2) disease, resulting in increased levels of IgE and eosinophilic inflammation of the airway. Asthma-like changes of the airways and lung parenchyma include eosinophilia, alternative macrophage activation, pulmonary lymphocytosis, mastocytosis, and epithelial cell proliferation with goblet cell hyperplasia and these are induced by Th2 cytokines (IL-4, IL-5, IL-9, and IL-13). The inhalation of allergens is known to activate T cells to produce a Th2 responses through stimulation of the innate immunity (Saenz et al. 2008; Otani et al. 2013). The increase in the levels of metalloproteinases in inflammatory condition has been documented. It has been reported that the levels of metallopeptidase 9 (MMP9) increase significantly in asthma (Okada et al. 1997). Probiotic bacteria including *Bifidobacterium bifidum*, *B. lactis*, and *L. lactis* are shown to inhibit Th2-related cytokines IL-5 and IL-13 and induces IL-10 significantly (Gorissen et al. 2014). In another review it has been shown that the probiotics consumption by expecting or nursing mothers or by infants reduces the risk of eczema in infants (Forsberg et al. 2016). Furthermore, short chain fatty acids generally produced by probiotic microorganism was also found to ameliorate airway inflammation in mice. The amelioration activity was dueto the decreased activity of T cells and dendritic cells, decreased numbers of CD4+ T cells producingIL-4, and reduced levels of circulating IgE (Cait et al. 2017). Convincing evidences are available in the literature to prove the role of gut microbiome dysbiosis in childhood Asthma. Depletion of symbiotic bacterial population such as *Akkermansia*, *Faecalibacterium*, and *Lachnospira* and an increase in certain fungi (*Candida* and *Rhodotorula*) in infants was found to be associated with the risk of developing atopy or asthma (Durack et al. 2018; Arrieta et al. 2015; Stokholm et al. 2018; Fujimura et al. 2016). The soluble proinflammatory products from the microbiome were shown to induce Th2 cells.

## *Probiotics and Central Nervous System (CNS)*

In addition to various health benefits of the probiotics clinical studies also suggest the role of gut microbiota on human brain development function (Tillisch 2014). As the brain and gut have a strong, two-way communication system which is often

referred to as the gut–brain axis. Probiotic dietary intervention of children with autism spectrum disorder (ASD) have shown improved performance of these children in schools (Umbrello and Esposito 2016). ASD children were shown to have some signature dysbiosis characterized by higher population of Bacteroidetes and Proteobacteria, and a lower abundance of Actinobacteria (especially *Bifidobacterium*) and Firmicutes (Finegold et al. 2010). This altered microbiome produced metabolites like significantly higher concentrations of ammonia that are considered neurotoxic and may further promote the adverse neurological effects associated with ASD (Wang et al. 2012; Morland et al. 2018). Furthermore, strains of *L. brevis* DPC6108 and *Bifidobacterium dentium* produced large amounts of the neurotransmitter γ-aminobutyric acid (GABA), which helps to suppress anxiety and depression (Barrett et al. 2012). Significant effect of probiotic intervention was observed in human subjects when single-strain probiotic including subspecies of *L. casei* (*rhamnosus*, Shirota), *L. plantarum*, and *B. infantis* were used for study (Rao et al. 2009).

## Fecal Microbiota Transplant (FMT): The Concept of Repoopulation

Many diseases especially those associated with GI tract, and conditions like irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) are due to the dysbiosis or the change in the gut microbiome. One of the recent approaches to treat these GI tract diseases is through Fecal microbiota transplant (FMT). The FMT is found to be especially effective in treating (>90%) recurring infections of antibiotic-resistant *C. difficile* infection (Smits et al. 2013). Based on such studies now FDA's guideline recommend the use of FMT for treating recurring *C. difficile* infection. As the name indicates FMT simply means the transfer of Fecal microbiota from a healthy donor to a diseased persons GI tract. The methods of transfer include nasogastric tube, nasojejunal tube, upper tract endoscopy and colonoscopy (Gough et al. 2011). Although it appears to be a new concept but it has been in use traditionally as it is reported that the Bedouins used to eat fresh camel feces to treat bacterial dysentery (Smits et al. 2013). The concept of FMT is gaining popularity as it is a very effective way of transferring a seed microbiome into a diseased person. Moreover, many microorganisms cannot be cultured under laboratory condition which makes it difficult to independently isolate these bacterial strains and then supplement these cultures as probiotic bacteria. The unknown composition of the Fecal microbiota is a matter of concern as the number and type of microorganisms in the samples to be transferred cannot be controlled and involves the risk of unexpected consequences. Although, the standard procedures involve precautionary measures such as screening of the donor's Fecal microbiota for transmittable diseases and fecal pathogens.

## Safety Assessment and Regulations of Next Generation Probiotic

Although probiotic bacteria are generally recognized as safe (GRAS), but there are genuine reasons to believe that probiotic strains should be evaluated carefully for their safety. One simple reason is the rapid industrialization of probiotic market and the increasing diversity of microorganisms that are used as probiotics. Many strains related to these probiotic bacteria such as the members of the genus *Clostridium* are pathogenic (Saarela 2019). Another property of probiotic bacteria which is often ignored is the fact that the genes for antibiotic resistance if present in probiotic strains may be horizontally transferred to the gut microbiome. Main risks involved with probiotics are possible infection and production of toxins by a contaminant strain or a misidentified probiotic strain. There are reports in the literature suggesting that the amendment of the diet with probiotics sometimes may result in disturb metabolism and in systemic infection (Doron and Snydman 2015). Therefore, it is necessary to ensure the safety and suitability of new probiotics. Some of the important properties that should be evaluated have been described by Saarela (Saarela 2019). The strain must be correctly identified using standard and modern molecular identification methods. A given probiotic preferably should not be related to any well-known pathogenic species. The whole genome sequencing should be carried out to check whether the strain contains antibiotic resistance genes and the strain should not have virulence genes. A candidate strain should not have enzymes involved in pathogenesis like collagenase, hyaluronidase and neuraminidase. The strains should also meet other requirements such as growth parameters and tolerance to gastrointestinal stresses. The strain should also exhibit technological feasibility for industrial production such as required growth in pilot scale and industrial scale fermenters. Candidate strains should also be evaluated in human studies for dose and other parameters.

Regulatory aspects involved in the industrial production of probiotic products such as efficacy, safety, and quality control as recommended by regulatory authorities must be followed. These regulations vary from country to country and so far, there are no universally agreed framework (de Simone 2019). Although, efforts have been made to develop such common guidelines and framework (Hill et al. 2014). European Union, regulate the probiotics under the Food Products Directive and Regulations. The EFSA (European food safety authority) has also allowed the use of some well identified strains as probiotics (Ricci et al. 2017). Under the European regulations any health claim for probiotics and probiotic based product has to be authorized by the EFSA. It is interesting to note that EFSA has rejected all submitted health claims for probiotics so far. In the USA also, the probiotic products are classified as foods or food supplements. And these products are required to comply with Good Manufacturing Practice (GMP) guidelines. It is allowed to make functional claims about the probiotic products in the United States. Nevertheless, the claims must not be misleading, and must be substantiated by scientific evidence (de Simone 2019). But the research on probiotics is regulated differently in

USA. Where, probiotics are categorized as drugs making it difficult to do much needed research on the probiotics (Hill et al. 2014). It is also important that the public health officers and medical professionals continue a post market surveillance of the products as recommended by FAO/WHO.

## Commercial Success of Probiotics

The recent commercial success of probiotics cannot be ignored as evident from the availability of various probiotic based products in the market. Some of these commercial dairy and non-dairy products include Yogurt, Kefir, Sauerkraut, Tempeh, Kimchi, Miso, etc. Web grab of some of these products are shown in Fig. 6. Especially the market of dairy products containing probiotic has grown tremendously (Stanton et al. 2001). According to the Journal of functional food the global market for probiotics has been estimated at US$33.19 billion in 2015. While, the value of functional food market in Europe alone in 1997 was at US$889 million. According to International Probiotic Association Europe (IPA, Europe) the sales of probiotic yogurt was valued at 5 billion Euro in 2016. It was found that UK and Italy were the largest consumer countries in Europe. On the contrary the market for probiotics was comparatively underdeveloped in USA by European standards. It has been pointed out in the report of IPA, Europe that the sales of the probiotic products have recently increased tremendously in North America and China. However, various new regulations on probiotics may adversely affect the market prospects. For example, it has been pointed out by the expert panel that new regulations of USFDA in this regard for probiotics will discourage much needed research on probiotics (Hill et al. 2014).

## Conclusions

The probiotic market is expanding rapidly throughout the globe and is a multibillion-dollar industry. This expansion is spurred by the better understanding of gut microbiome and its health benefits. With the advancement of science and technology, intervention trials, meta-analyses and systematic reviews convincing evidence of the health benefits associated with probiotics have been gathered. It has been proved that probiotics can effectively treat various GI tract associated diseases and disorders the most common being different types of diarrhea, enterocolitis and IBS. The new FDA guidelines to treat recurring infection of *C. difficile* recommend the use of Fecal microbiota transplant. It is also claimed that probiotics can help in controlling diabetes, cancer and obesity. For many of such claims molecular markers have been identified. Though it is difficult to understand the molecular basis of some of these claims especially of mixed strain probiotics or undefined community such as Fecal microbiota. While the mechanism of others is much simpler to under-


**Fig. 6** Variety of the commercial products available in the market. These include dairy products like milk, yogurt, ice cream, soft drinks, fermented foods like pickles and health supplements

stand such as the production of lactase which helps in lactose digestion. Notably, regulatory authorities have not approved any health benefit claims of probiotics so far. Expert committee has also recommended that robust evidences must be provided to claim any health benefit associated with a candidate probiotic. Although it is accepted by the expert committee that these microorganisms have some core health benefits.

The commercial success of the probiotics has also led to an increased interest of the scientists in probiotics resulting in the discovery of many new and nonconventional probiotic microorganisms referred to as next generation probiotics in this book chapter. These microorganisms have been isolated from a variety of sources such as dairy products, conventionally used fermented foods, fecal matter, human milk etc. The discovery of these microorganisms and many more in near future would require clear guidelines to correctly identify a candidate strain. Since, the direct consumption of live cultures is involved any misidentified strain may result in infection and disease. Therefore, stringent regulations for probiotics are required to protect the interests of producers as well as of other stake holders. These regulations should be aimed at controlling the misuse of the term probiotics and protecting the consumers from any potential harm of misidentified products. For example, the dispersal of antibiotic resistance genes through probiotics should be checked carefully. The unfounded claims should be discouraged. The new candidate strains must be correctly identified using modern and conventional techniques and must be evaluated for the presence of the core health benefits. It is also recommended that these strains should be submitted to at least two international culture collections. The use of undefined microbial communities such as the Fecal microbiota from healthy individual though have clear benefits but technically cannot be categorized as probiotics. The use of such undefined microbial communities is always risky and require proper screening and control measures to minimize the risks involved.

However, on the other hand framed regulations should not discourage research in the field which may deprive the consumers from any future benefit of the probiotics. As the expert panel have pointed out that the classification of probiotics under the "drug" category for research in the USA may discourage the much-needed research in this area. Therefore, regulations on probiotics must be balanced and in the interest of a sustainable development of the probiotic market. In fact, this will require a close communication and collaboration between academics, health professionals, industry, regulatory agencies, and policy makers. Furthermore, the regulations for the probiotic products vary from country to country resulting in confusion. A consensus is required for the smooth and global implementation of these regulations. It is also equally important to have regulations for post-production follow up to ensure the safety and effectiveness of the products by an industry. Post/parabiotics which are the metabolic by-products, dead microorganisms, or other microbial-based products are comparatively safer but cannot have long term effects that are obtained through the establishment of probiotic bacteria in the gut.

Probiotics hold great health benefits and in future can play a bigger role in treating and preventing various diseases especially those caused by dysbiosis. Since, much progress is already made in understanding the role of these microorganisms

in treating various diseases. These microorganisms can also be customized to deal with specific health issues. For example, the role of *Akkermansia muciniphila* in controlling obesity, Fecal microbiota transplant in treating *C. difficile* infections, *Lactobacillus* in treating different types of diarrhea and role of *Bifidobacterium* spp. in prevention of type 2 diabetes and obesity is very clear. It is also suggested that various probiotic strains can be genetically modified for improving their potential as probiotics (Ahmed 2003). Scientists are even checking the survival of these probiotics in international space stations (Sakai et al. 2018). Although, the understanding of probiotics and the associated health benefits has improved significantly a lot still needs to be done. Especially through successful isolation of various strains in monocultures that will also make it easier to understand the potential health benefits of these strains and the mechanism involved.

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