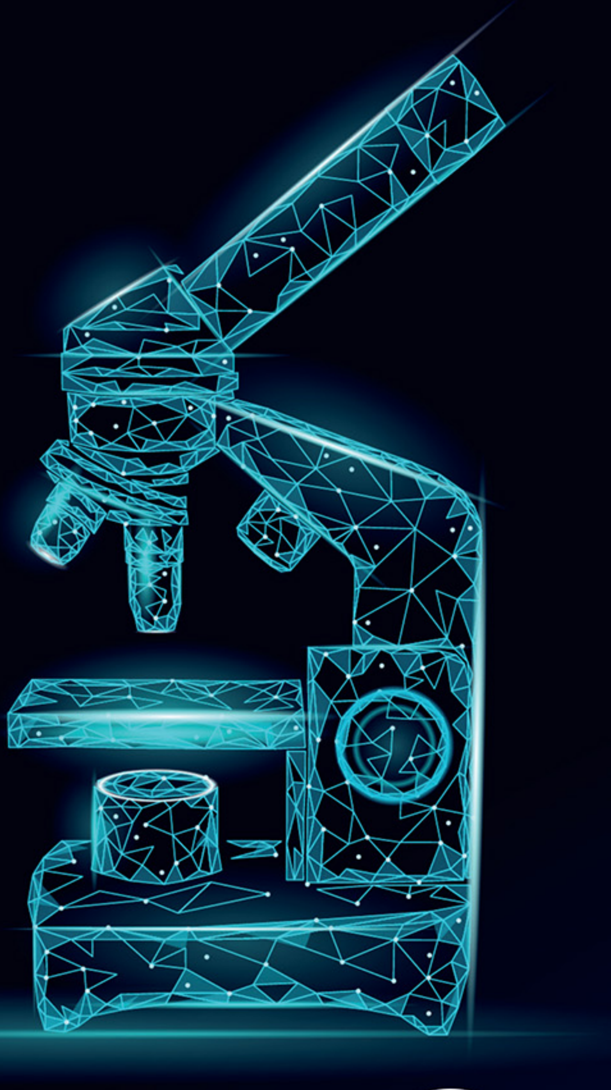
Probiotics in the Prevention and Management of Human Diseases

A Scientific Perspective



Edited by
Mitesh Kumar Dwivedi, PhD
N. Amaresan, PhD
A. Sankaranarayanan, PhD
E. Helen Kemp, PhD

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Mitesh Kumar Dwivedi
C.G. Bhakta Institute of Biotechnology, Faculty of Science,
Uka Tarsadia University, Maliba Campus, Bardoli, India

N. Amaresan
C.G. Bhakta Institute of Biotechnology, Faculty of Science,
Uka Tarsadia University, Maliba Campus, Bardoli, India

A. Sankaranarayanan
Department of Life Sciences, Sri Sathya Sai University for Human Excellence,
Gulbarga, India

E. Helen Kemp
Department of Oncology and Metabolism, University of Sheffield, Sheffield,
United Kingdom

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ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-823733-5

For Information on all Academic Press publications
visit our website at https://www.elsevier.com/books-and-journals

Publisher: Charlotte Cockle
Acquisitions Editor: Megan Ball
Editorial Project Manager: Moises Carlo Catain
Production Project Manager: Sruthi Satheesh
Cover Designer: Victoria Pearson Esser

Typeset by MPS Limited, Chennai, India

Chapter 29

Probiotics: past, present, and future challenges

Marieta Georgieva, Kaloyan Georgiev and Nadezhda Hvarchanova
Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

29.1 Probiotics—the concept

Diseases existed long before evolution reached *Homo sapiens*. Over thousands of years, man has been immersed in an ocean of microorganisms invisible to him, which not only made him ill, but also helped him heal and survive. Only in the 19th century, called the "golden age of microbiology," did the great researchers R. Koch, L. Pasteur, I. Metchnikoff and others point out that there are pathogenic microorganisms visible under a microscope which are "enemies" of human health. Yet many centuries before these epoch-making discoveries, people knew about the beneficial effects of fermented foods; contemporaneous probiotics are derivatives of these ancient food stuffs. The use of fermented milk is a part of the history of human civilization. The term "fermentation" has a Latin root (fermentum—boiling), as the grapes crushed for wine have formed bubbles, characteristic of boiling liquid. The use of fermented milk has been known since ancient times (Sumer, Egypt, Greece, and Rome). Geographical differences and cultural traditions have coined its name: koumiss, kefir, yogurt, etc. The secret of fermentation was revealed by Louis Pasteur (20th century), who states that the process is due to the presence of yeasts, but does not make an association between these microorganisms and their possible health effects.

Professor I. Metchnikoff took the next big step in 1907 (Metchnikoff, 1907). He formulated the hypothesis of putrefactive microorganisms inhabiting the human colon, which leads to a kind of autointoxication with waste metabolites and thus shortens human life. Shortly before that (1905) the young Bulgarian doctor Stamen Grigorov discovered in Geneva the lactic acid bacillus, which leads to fermentation of milk and turns it into yogurt; he worked with an lactic acid starter sent to him directly from Bulgaria. Metchnikoff believed that *Lactobacillus bulgaricus* suppressed putrefactive pathogens in the colon and thus helped to prolong human life (the scientist himself consumed yogurt daily for the rest of his life). That marks the beginning to many studies on the healing and prophylactic properties of yogurt and *Lactobacilli*.

Two world wars and the discovery of antibiotics overshadowed Metchnikoff's ideas for decades. Yet in 1965, the name "probiotic" entered the medical literature and has no longer been off the stage. Of 176 publications in literature, there were 1476 (2014) articles and 477 randomized (medical) trials (according to PubMed). In February 2019, the number of publications related to probiotics was 20,315, which illustrates the great interest and human hope for modern application of lactic acid fermentation products.

Probiotics are a concept from the beginning of the 20th century, which modern humans need more and more frequently. Furthermore, today they are accepted and scientifically proven to be a useful component in nutrition, with an important role for good physiological status, healthy balance and disease prevention in humans and animals. The word "probiotic" comes from Latin ("pro" meaning for), and ancient Greek ("bios" meaning life), that is, "for life." It was first used in 1954 to compare the harmful effects of antibiotics with those beneficial to the bacteria probiotica. Today, the International Scientific Association for Probiotics and Prebiotics defines "probiotics" as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014). Probiotics as "living microorganisms" have prophylactic and therapeutic effects that improve the internal microbial balance.

Today, the world is returning to natural remedies for health prevention. Probiotics take the leading place among them. It creates a new challenge for scientists, technologists, and physicians. A response to new requirements in the

Probiotics in The Prevention and Management of Human Diseases. DOI: https://doi.org/10.1016/B978-0-12-823733-5.00025-8

21st century is the elaborateness of new technologies for the production of probiotics, and functional foods for the prevention and treatment of many diseases that lead to the genesis of the disturbed balance of gut microbiota (Anukam & Reid, 2007; McFarland, 2015).

The concept of functional foods was first created in Japan in 1980 (The American Dietetic Association). Functional or "biotherapeutic" foods include specific additives and substances that have a positive effect on human health, determine good health and, along with their nutritional qualities, contribute to the prevention of certain diseases (Salminen, 1996). Certain natural products can be classified as functional if they contain a nutritional component that positively affects any of the physiological functions in humans and/or has a protective, healing effect. Functional foods are various products containing probiotics, prebiotics, vitamins and, to some extent, specific supplements. The most significant and well-known group among them are probiotic foods containing live bacteria that have a positive effect on the human body. They have been shown to have a better health effect than traditional foods and most often this is directly related to the available living microorganisms with unique biological properties (Salminen & Marteau, 1997). Consequently, probiotics and products with their participation are gradually becoming an important element of the food chain and as such need to be monitored and evaluated very seriously.

New challenges in probiotic research include the creation of modern forms of food, supplements, and therapeutics, meeting the requirements of reliability—safety, convenience and usefulness, according to European regulatory frameworks—as well as providing knowledge about the genetic basis and molecular mechanisms of action to ensure optimal health for each individual. This will allow the creation of the so-called. targeted probiotic preparations that will best meet the needs of the target groups of consumers without risk in their application.

29.2 Probiotics—modern trends

29.2.1 Definition and classification of probiotics

Recent decades marked an evolution with the discovery that the human gastrointestinal tract is a host for a huge number of beneficial bacteria known as the intestinal microbiota. It includes at least 500−1000 species in each person, with about 100 trillion cells, which exceeds 3−10 times the number of human cells (Guarner & Malagelada, 2003; Human Microbiome Project Consortium, 2012). Bacteria make up 90% of human microbiome (HM) and are the best studied microorganisms in the intestinal ecosystem. With the development of high-performance gene sequencing technology, the study of HM currently includes two main steps: (1) 16S rRNA-based bacterial gene sequencing and (2) bioinformation analysis. Metabolomics is another rapidly evolving field of HM research that evaluates small molecules associated with the interaction between human metabolism and bacteria, which has an impact on health and disease. Data from the study of HM and metaboloma currently provide the strongest evidence for their association with health and various diseases (Cani & Delzenne, 2011; Dave et al., 2012; Lloyd-Price et al., 2016). In addition, the genes encoded by the bacterial genome are about 150 times the genes encoded by the human genome. These additional genes have added various types of enzymatic proteins that play an important role in facilitating the body's metabolism and thus contribute to the regulation of human physiology (Hooper & Gordon, 2001; Ursell et al., 2014).

For years, medicine has been fighting infections with the help of antibiotics and the lives of millions of people have been saved thanks to them. Today we know, however, that their use leads to an imbalance in the intestinal microbiota of the body, which has adverse consequences. Awareness of the importance of the microbiota for health has led to its largest study to date, the American Human Microbiome Project. That project, which lasted 5 years (2007−12) (Human Microbiome Project Consortium, 2012), aimed to study in detail the whole set of microorganisms inhabiting the human body—the so-called human microbiota—and collect complete information about their genetic material: the HM. As a result, a map of the human microbiota inhabiting the mouth, upper respiratory tract, gastrointestinal tract, urogenital tract, and skin was created. It turns out that we coexist with single-celled organisms that exceed the number of our own cells in a ratio of 10:1, that is, man is a macro-organism with 10 times more belonging to microorganisms.

The list of global microbiome studies is becoming increasingly impressive. In 2012, the Canadian Microbiome Initiative was launched, the European Metacardis project, mainly related to cardiovascular diseases, as well as the MegaHit project that implemented in collaboration with China. China is independently running an autonomous project called MegaGut, while Japan is focused on the Human Metagenome Consortium. This steady increase in global microbiome research projects is actually a logical echo of the human genome sequence we witnessed in the 1990s (Araya et al., 2002).

Each person has a unique microbiota, which they acquire at birth. It changes with age, health, and lifestyle. Its composition includes bacteria, fungi, and viruses that play different roles in our body. They help digest and assimilate food, communicate with the host's immune system, and serve as a shield against unwanted environmental pathogens. In turn, the human body offers them habitat and a nutritious environment.

These mutually beneficial relationships are fragile and the balance can easily be upset. Changes in the environment associated with malnutrition, decreased immunity, stress, and various diseases, lead to quantitative and qualitative changes in the microbiome. The huge differences in the modern way of life compared to the way of life of our ancestors barely a century ago have brought about drastic changes in the microbiome that has evolved with us for millions of years. Scientists link these changes to some of the most common chronic and autoimmune diseases today. More than ever, the role of microorganisms in human life needs to be rethought (Araya et al., 2002).

With the development of research on probiotics, the need for them to be correctly defined and validated increases. At the request of the Argentine government, in 2001 the FAO and WHO sponsored an expert advisory group. During the consultation, which was chaired by Dr. Gregor Reid, Director of the Canadian Center for Probiotics Research and Development, the group adopted by consensus the definition of "probiotics" as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." Thus in 2002, the WHO officially defined probiotics. (Araya et al., 2002; Hansen, 2002). This is currently the most widely used and accepted definition, as it covers all applications of probiotics as live microbes, not just the benefits of intestinal homeostasis. Two main features are considered to be the most important:

1. Probiotic preparations must contain live microorganisms.
2. The desired effects should be related to the amount taken.

The main probiotics used in the present commercial preparations contain lactic acid bacteria (LAB), including *Lactobacilli*, and *Bifidobacteria* (Hansen, 2002).

The European institutions have the following requirements for functional foods:

- to be taken daily;
- be part of normal food;
- be obtained from natural products;
- have a positive healthy effect on the body; and
- reduce the risk of disease and improve the quality of life.

The effectiveness of probiotics depends on many factors: their composition, the state of the microbiome in the body, as well as the age, gender, and living conditions of the individual.

In general, probiotic strains can be divided into two groups:

1. resident
2. transiting

The strains of the first group are permanent and often found in the GIT. Preparations based on such microorganisms favor their recovery in the intestinal tract. Resident strains are more compatible and less antagonistic to other beneficial microorganisms permanently present in the human body; it is a kind of evolutionarily fixed microbial association with a positive effect on the host organism. The following species have been described as resident in humans: *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Lactobacillus crispatus*, *Bifidobacterium* spp. Transiting strains belonging to the species *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus yogurti*, *Lactobacillus brevis*, *Lactobacillus kefir*, *Lactobacillus plantarum*, only temporarily reside in the intestinal tract, but during the transition can also have a positive effect. Therefore the use of multistrain preparations containing bacteria from both groups is recommended. Only individual strains meeting certain selection criteria are used in practice, however.

The International Research Group on New Antimicrobial Strategies (ISGNAS) is developing a concept for a detailed definition of probiotics in the following three categories (Rusch, 2002):

1. Medical probiotic—microbial preparation containing live and/or killed microorganisms, including their components or metabolites, formulated for therapeutic use, as a medicament for treatment and prophylaxis.
2. Pharmaceutical probiotic—a microbial preparation designed as a food supplement.
3. Food probiotic—a microbial preparation designed for use in food fermentation and food production. The mechanism of action of which includes immunomodulation, effective influence of the protective microflora and modulation of metabolic activity (Rusch, 2002).

This division, although still debatable, gives a more accurate picture of the scope of probiotics and can be adopted with a view to drafting legislation and regulations on probiotics.

Probiotics can be considered in groups according to the field of application and the host:

- Probiotics for stock-breeding.
- Food probiotics and food supplements for humans.
- Probiotics with a medical focus for prevention and treatment, which may include not only live microorganisms but also their active metabolites or components.

Regardless of their subdivision, both scientists and food experts on food safety and risk assessment are consolidating around the attitude that should be proven for each proposed probiotic:

- origin and identity;
- safety; and
- efficiency and effectiveness—that is, to demonstrate the physiological benefits of using a number of viable probiotic bacteria in the appropriate forms of administration (food, capsules, etc.) in a number of placebo-controlled patients and/or a standard treatment option if the final result is treatment of the disease.

These are the main indicators by which the practical acceptability of each probiotic is assessed today and they are complementary, but no less important, criteria. The next step in the development is to include these biologically active microorganisms and/or the active metabolites produced by them in the production of naturally protected and functional foods, probiotic food, and feed additives or in formulations with therapeutic applications.

Prospects for the development of probiotic products are closely dependent on scientific evidence of their safety, organoleptic properties, health, and therapeutic effects (Ward & German, 2004).

More detailed studies of the main groups of probiotic microorganisms (lactic and bifidobacteria, as well as some yeasts) and new biomarkers are needed to adequately reveal the physiological potential of functional foods and the lactic acid microflora involved in their production (Mattila-Sandholm et al., 2002).

29.2.1.1 Criteria, selection, and application of probiotic strains

The selection of probiotics includes two types of analysis:

1. in vitro analysis, which are important for the overall selection process and for the preselection from a large number of strains of potentially probiotic ones. This type of analysis is based on the use of different model systems.
2. controlled in vivo studies in healthy volunteers given a specific dose of a strain or strains selected by in vitro analysis (Morelli et al., 1986).

The direct addition of probiotic microorganisms to functional foods, nonpharmacological or medicinal products require a careful selection of strains suitable for industrial use. Their qualities and functional properties must be established. Three main aspects can be formulated that should be taken into account when selecting probiotic strains: (1) safety, (2) functionality, and (3) technological ability.

29.2.1.2 Characteristics of probiotic strains

Probiotics are determined by their specific strain, which includes the genus, species, subspecies (if applicable) and the alphanumeric designation of the strain (Guarner et al., 2017). The seven main genera of microbial organisms most commonly used in probiotic products are *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia* and *Bacillus*. Table 29.1 shows examples of the nomenclature used for several commercial strains of probiotic organisms.

TABLE 29.1 **Nomenclature for sample commercial strains of probiotics.**

Genus	Species	Subspecies	Strain designation	Strain nickname
Lactobacillus	*rhamnosus*	None	GG	LGG
Bifidobacterium	*animalis*	lactis	DN-173 010	Bifidus regularis
Bifidobacterium	*longum*	longum	35624	Bifantis

Lactobacillus and *Bifidobacterium* are the two main genera of Gram-positive bacteria widely used as probiotics. They are the two well-studied probiotic strains recommended for improvement of gut health and gut immunity (Sanders, 2008; Vajro et al., 2014). LAB are the most commonly used microorganisms as probiotics that can be isolated from humans, animals, plants, and the environment. The most common microorganisms used in the preparation of probiotics are those associated with the human gut flora. These include mainly LAB, *Bifidobacteria* spp. and *Propionibacteria* spp. They are often found in fermented foods, especially dairy products. They are quantitatively the most important ingested bacteria. Therefore most studies focus on the effects of these bacterial groups in probiotics on the gut microbiota and the health of the host (de Vrese & Schrezenmeir, 2008; Singh et al., 2013).

29.2.1.3 Lactobacillus bulgaricus—*a unique probiotic*

Lactobacillus bulgaricus is the only probiotic named after a specific people and geographical area. It is the first microorganism selected by man thousands of years ago and changed its evolution. *Lactobacillus bulgaricus* is of plant and aquatic origin and is isolated from green plants only on the territory of Bulgaria. Once in other parts of the world, it mutates and stops reproducing (Fig. 29.1A and B).

The territory of Bulgaria is host to the plants draca (*Paliuris aculateus*), sour (prickly) thorn (*Berberis vulgaris lam*), from the flowers of dogwood (*Cornus mas* L), the flowers of the Bulgarian rose (*Rosa damascena* var *trigintipetala*), in the roots and bark of oak trees (*Quercus robur* L).

Rods were isolated from *Calendula officinalis*, *Cornus mas*, *Galanthus nivalis*, *Prunus spinosa* and other unidentified plant species. Cocci were isolated from *Calendula officinalis*, *Capsella bursa-pastoris*, *Chrysanthemum*, *Cichorium intybus*, *Colchicum*, *C. mas*, *Dianthus*, *G. nivalis*, *Hedera*, *Nerium oleander*, *Plantago lanceolata*, *P. spinosa*, *Rosa*, *Tropaeolum*, and others. Both rods and cocci were simultaneously isolated from *C. officinalis*, *C. mas*, *G. nivalis*, and *P. spinosa* (Michaylova et al., 2007).

During the spring days, in the beginning of may, *L. bulgaricus* can be isolated from spring water in oak forests. With this spring water it is possible to ferment milk. These springs have been defined by the Bulgarian population as sacred for hundreds of years. For the first time in the world, in 2011, Bulgarian scientists (N. Alexandrov and D. Petrova) managed to isolate eight completely natural, original strains of *L. bulgaricus*, *Lactobacillus helveticus*, *Lactobacillus lactis*, and *Streptococcus thermophilus* from spring water in the Balkan Mountains, near a Thracian settlement (Fig. 29.1A and B). Using the method of modern biotechnology, these scientists managed to create a new probiotic formula, as an established probiotic cocktail (LDB-ST). All probiotics containing strains of aquatic origin are patented in the United States with an official patent number in 2015 (US Patent, 9,131,708B2/2015, September 15). Due to their all-natural origin, all these new strains have maximum resistance and survival when passing through the gastrointestinal tract and retain their number and viability under normal storage conditions for up to 2 years. (Georgiev et al., 2015).

It is well known that ST, an established yogurt-producing bacterium, is useful for improving and maintaining health, along with the protooperative LDB bacteria (Herve-Jimenez et al., 2009). LDB and ST work closely together, with the presence of ST stimulating the growth of LDB through the production of formic acid and carbon dioxide, while LDB promotes the growth of ST by providing the necessary amino acids by breaking down proteins (Driessen et al., 1982; Veringa et al., 1968). It has been reported that this cooperation between LDB and ST is one of the reasons for the production of products with specific qualities that cause effects in vivo and are clinically active against pathogens (Carper, 1994).

Probiotics belong to the GRAS (Generally Recognized As Safe bacteria) group, according to the US Food and Drug Administration (FDA). The experience gained by man over the millennia, as well as modern scientific research, show that the LAB used are harmless to the human and animal body.

29.3 Viability of probiotic bacteria in the gastrointestinal tract and their secondary reproduction: probiotic concentration

Oral probiotic bacteria are exposed to various factors—saliva, gastric juice, bile acids, etc.—which initially aim to stop the entry of various bacteria into the body. In addition, they are affected by food, water, beverages, and other microorganisms. The ability of probiotic bacteria to stay alive in sufficient quantities as they pass through the upper sections of the digestive system is one of their most important requirements. Not all types of probiotic bacteria have qualities that allow them to overcome these barriers. Probiotic bacteria are known to have a higher survival rate when taken as part

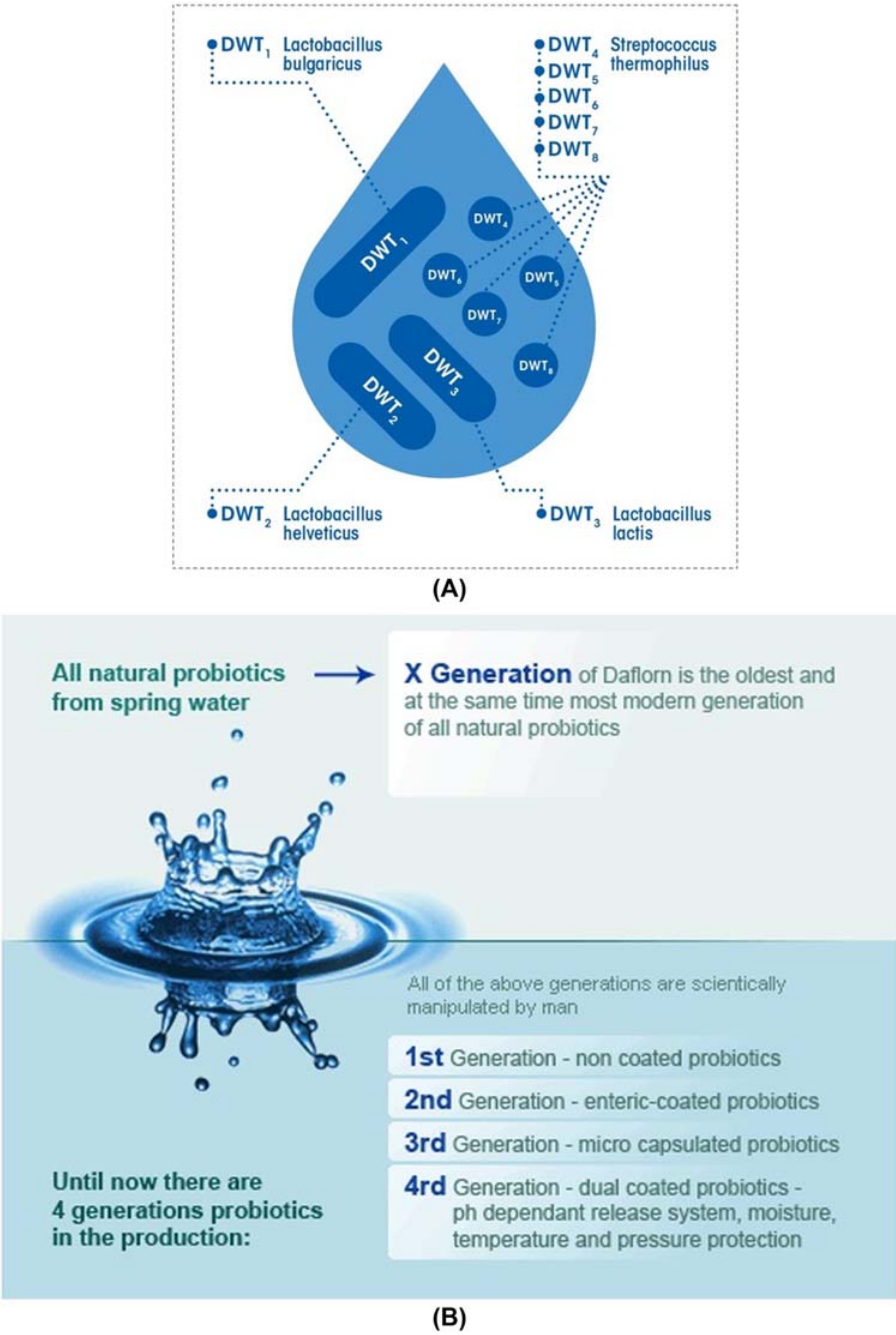

FIGURE 29.1 Probiotics from spring water: (A) all isolated probiotics from spring water; (B) generations.

of a lactic acid product than as a pure culture (Georgiev et al., 2015). The use of modern biotechnological processes for the production of new probiotic lactic acid products achieves a significantly higher survival rate of beneficial bacteria.

Different methods for quantifying probiotic bacteria have been established in different parts of the world.

In the countries of the European Union, a method for counting the number of live probiotic microorganisms in solid nutrient media at the time of sowing has become widespread. To define a product as probiotic, it must contain more than 10,000,000 (107) live probiotic microorganisms in 1 g of probiotic product. In the United States, Canada, Japan, Korea, and other countries, a method is used to enumerate colony-forming units (CFUs) in a liquid medium at 72 hours after inoculation. In Russia, a method is used to quantify probiotic bacteria as a weight fraction of 1 g milligrams of probiotic bacteria in 1 g of probiotic product. This leads to different results.

Mass manufacturers of probiotic products write on the labels the number of living cells or CFU at the date of manufacture. Their products should be stored in the refrigerator, because if they are not wrapped naturally or synthetically. The number of living cells in the products for several months decreases rapidly and then their health effects are minimal. Leading French companies in the field of probiotics are developing methods for wrapping probiotic bacteria with synthetic proteins.

29.4 Dose of probiotics

The beneficial effect of probiotic bacteria on the human body is associated with their intake in sufficient quantities. There is currently no consensus on the required single or daily dose. It is assumed that the amount of living cells of a certain type and strain should be consumed in order to obtain a clear health effect. Most authors assume that this amount should be between 3 and 5 billion living cells per day (Lee & Salminen, 2009).

Gibson et al. differentiates that if probiotic bacteria are taken in lactic acid products, their amount should be 2−3 billion per day, and if taken as pure cultures (only live cells), from 10 to 15 times more (15−30 billion) (Gibson et al., 2010). In some European countries, probiotic bacteria are thought to have minimal health effects at doses above 1 million. Studies show that this amount is too small to have a distinct health effect, and the daily dose should be significantly higher, on the order of several billion living cells. As mentioned, the health effects of probiotic bacteria depend on a number of factors, such as resistance to passing through the upper sections of the digestive system, the type of strain, and so on (Gibson et al., 2010).

29.5 Safety of probiotic bacteria

One of the main requirements for probiotic bacteria is that they are safe for the consumer. Intake of probiotic LAB by children, pregnant women, or the elderly is not considered a risk factor. Moreover, a number of observations and clinical studies have shown that taking probiotics strengthens their immune system and improves their health.

It is essential that the safety assessment of bacterial strains used as probiotics is thoroughly studied. Probiotics belong to the GRAS group, according to the FDA. The experience gained by man over the millennia, as well as modern scientific research, show that the LAB used are harmless to the human and animal body. They belong to the group of GRAS microorganisms, that is, generally accepted for safe bacteria. There is also no evidence that taking them in larger doses than usual will lead to side effects (Salminen & Marteau, 1997). However, both WHO and FAO stated that probiotics may results in several types of adverse events, at least theoretically. Microorganisms used as probiotics can cause systemic infections, stimulate the immune system, disrupt metabolism, and participate in horizontal gene transfer. Brown & Valiere (2004) and Salminen (1996) reported some methods for assessment the safety of LAB, using in vitro studies, experimental animal tests, and clinical trials. Their combination has proven that most of the current probiotic strains meet the required safety standards. According to Salminen and Marteau (1997) further studies are needed on the characteristic properties of probiotics, their pharmacokinetics, and the interactions between them and the host in order to assess their safety.

29.6 Health effects of probiotics

29.6.1 Prevention and treatment with probiotics

Probiotics play a responsible role, especially in the prevention of a large number of serious diseases, originating from a disturbed balance of the GIT microbiota such as gastrointestinal infections, dysbacteriosis, enteritis, gastritis, ulcers, high cholesterol, weakened immune system, tumors, and others. Through appropriate prevention, these diseases can be brought under control and society can save a huge resource (labor, material, financial, mental). Active scientific disclosure of the positive results of the evaluation of a large number of probiotic crops and the opportunities they provide to the health concerns of the consumer lead to increasing trust and increased demand. Not coincidentally, probiotics and probiotic foods are among the most sought after on the market in Europe.

The economic importance of probiotics is great: the global market for probiotics amounted to US$40 trillion in 2017 and is expected to grow to US$64 trillion by 2023 (Reid et al., 2019). Europe is a traditional market leader in

LAB with probiotic properties and mainly *Lactobacilli*. *Lactobacillus* and *Bifidobacterium* are the two main types of Gram-positive bacteria widely used as probiotics (Araya et al., 2002).

In today's global society, where people are exposed to daily stress, the widespread use of antibiotics, poor nutrition, and a number of harmful external factors, the balance of the gastrointestinal flora is constantly disturbed. The ability to control and predict the effects of probiotics based on rigorous research would allow both consumers and manufacturers to choose and offer products with proven curative properties and thus fully extend human life.

Understanding the mechanisms of probiotic bacteria action is perhaps the biggest challenge before us. There is currently very little information about the mechanisms by which probiotics provide their health effects. Probiotic strains are living microorganisms that most likely work through multiple mechanisms and molecules, yet there is a real need for more in-depth research. Researchers are trying to scientifically explain how probiotics work should be applauded, which will allow for more rational approaches to the selection and use of probiotics.

As you can see in Table 29.2, a significant number of health effects have been found as a result of taking probiotics: relieving lactose intolerance, stimulation of intestinal immunity; prevention of pathogen colonization; lowering serum cholesterol and blood pressure, reduction of inflammatory reactions, cancer prevention, reduction of food allergies, modification of the intestinal flora, and improving well-being. (Simmering & Blaut, 2001). Much of the research proves the essential role of probiotics in proper digestive function and healthy balance in the GIT and urogenital tract. At the same time, scientists focus on the functional effects of foods and their health properties associated with bioactive and probiotic crops (appetizer and/or supplements). Functional foods provide the body not only with essential nutrients (Table 29.3), but also with healthy bioactive components.

Recent research shows that people with psychoneurological diseases (depression, chronic fatigue, and other mental illnesses) suffer from severe dysbacteriosis. Correction of dysbiosis with probiotics has a beneficial effect on the underlying disease (Bercik et al., 2012; Boukthir et al., 2010; Kennedy et al., 2012). In the last year, a new challenge has emerged: The use of probiotics to prevent Covid -19? It is worth to be noted that probiotics, although harmless, are not a magic panacea. Continuous and adequate supervision of their use is necessary to prevent even the slightest risk to human health.

In recent years, probiotics have been studied as natural living carriers of vaccines, enzymes, antimicrobials, as they are biologically active molecules with a number of health and nutritional effects.

Further application of advanced "-omics" technologies will provide a better understanding of complex host-bacterial interactions, but research strategies need to be well planned to provide informative data that can be interpreted biologically. Once these biological mechanisms and physiological interactions are clarified and more confidently defined, the

TABLE 29.2 Health effects of probiotics.

Effects	Probable mechanism
1. Help digest lactose	Bacterial lactase breaks down lactose in the small intestine.
2. Resistance to internal pathogens	Auxiliary effect, increasing the production of antibodies. Systemic immune effect. Resistance to colonization. Creates unsuitable conditions for pathogens.
3. Antitumor effect of the colon	Antimutagenic activity. Alters the procarcinogenic activity of colonizing microbes. Stimulates immune function; Reduces the concentration of bile salts.
4. Reduce the overgrowth of pathogens in the small intestine	Lactobacilli affect the activity of overgrowth. Reduce toxic metabolites.
5. Modulation of the immune system	Auxiliary effect in antigen-specific immune response. Cytokinin production.
6. Antiallergic effect	Prevents translocation of antigens in the bloodstream.
7. Antisclerotic action	Assimilation of cholesterol by bacterial cells. Antioxidant effect. They change the hydrolase enzyme activity.
8. Antihypertensive effect	Cell wall components act as ACE inhibitors.
9. Infections caused by *Helicobacter pylori*	Competitive colonization.
10. Hepatic encephalopathy	Inhibit urease-producing flora.
11. Urogenital infections	Reduce bacterial adhesion to the urinary and vaginal tract.

ACE, angiotensin-converting enzyme.

TABLE 29.3 Nutritional effects of probiotics.
Increase the absorption of proteins, fats and carbohydrates.
Increase the absorption of minerals and trace elements—calcium, potassium, phosphorus, iron, magnesium, selenium, zinc.
Increase the synthesis and stabilize vitamins—A, B1, B2, B6, B12, C, D, E.
Increase and stabilize the enzymes protease, lipase, amylase, cellulase.
Increase synthesis and stabilize hormones.
Stimulate the secretion of digestive juices.
Stimulate growth.

extrapolation from laboratory science to clinical treatment with probiotic supplements will be significantly accelerated. The future of probiotic research offers exciting potential to influence human health and disease.

29.6.1.1 Probiotics and diseases of the gastrointestinal tract

The 2005 Nobel Prize in Physiology or Medicine by Robin Warren and Barry Marshall reminds us that the solution to some diseases lies not only within the human body, but rather can be found on the border with the microbial environment. Manipulation of CM (intestinal microbiota) is becoming a realistic therapeutic and prophylactic strategy for many infectious, inflammatory and even neoplastic diseases in the intestine, as well as for diseases outside the GIT. However, the promise of pharmabiotics is unlikely to be fulfilled without paying more attention to the secrets hidden in the neglected internal organ (the GIT). It is a rich repository of metabolites that can be used for therapeutic purposes. Therefore the deep penetration into the molecular details of the interactions between the organism and the intestinal microbiome is a prerequisite for the discovery of new therapeutic possibilities for many diseases. A number of stomach ailments, such as gastrointestinal infections, Crohn's disease, ulcerative colitis, food allergies, antibiotic and infectious diarrhea, all benefit from probiotics (Derwa et al., 2017; Kalliomäki et al., 2001).

One of the most thoroughly researched areas for probiotic use is the prevention of antibiotic-associated diarrhea (AAD). It is important to note that not all probiotic strains will be as effective for this purpose. For example, a recent systematic review comparing the efficacy and tolerability of different probiotics for AAD, where the authors examined 51 RCTs ($n = 9569$) and found that treatment with *Lactobacillus rhamnosus* GG (LGG) was significantly better than that of any other strain of AAD when used to prevent this condition. While *Lactobacillus casei* has a higher efficacy (Cai et al., 2018) in reducing the rate of *Clostridium difficile* infection, it should therefore always be considered which probiotic will be used, depending on the patient's condition and the desired outcome. Another condition of the gastrointestinal tract in which probiotics have been shown to be beneficial is the colonization of *Helicobacter pylori*, which is a problem for about 50% of the world's population (Brown, 2000).

29.6.1.2 Hepatoprotective effect of probiotic bacteria

Toxic liver damage is of increasing interest due to its increasing frequency. The most widespread worldwide are drug-induced and alcoholic hepatotoxicity. Drug-induced hepatotoxicity is a leading cause of acute liver failure in Western Europe and the United States, associated with involuntary or targeted acetaminophen overdose (Suk & Kim, 2012; Yoon et al., 2016).

Standard therapy for liver disease in many cases is insufficiently effective and safe for the patient. The addition of probiotics can significantly improve the treatment of patients suffering from toxic liver damage, as well as to be used in the prevention of liver disease. From a physiological point of view, the close functional and two-way communication between the intestine and the liver is one of the most important connections between the HM and the extraintestinal organs. Due to its unique anatomy and vascular system, the liver receives about 70% of its blood supply from the intestines through the portal vein. Thus it is constantly exposed not only to the products of digestion and absorption, but also to adverse substances from the intestinal lumen derived from the intestine, including bacteria and bacterial components such as lipopolysaccharide (endotoxin) 23 (Ponziani et al., 2018). The liver is an important immunological organ and after the entry of these substances through the portal circulation, it reacts by activating the innate and adaptive immune system with its subsequent damage. A better understanding of the pathophysiological links between intestinal dysbiosis, intestinal barrier integrity, and hepatic immune response to intestinal-derived factors is essential for the development of new therapies for the treatment of chronic liver disease (Tripathi et al., 2018).

Experimental and clinical studies have demonstrated the possible use of probiotics to prevent liver damage, and that the probiotic may be an effective hepatoprotector in the treatment and prevention of liver disorders (Georgieva, 2006; Iannitti & Palmieri, 2010).

The main attention is paid to the mechanisms of the hepatoprotective effect. According to the literature, understanding these mechanisms is of paramount importance in the future, as a number of liver disorders (inflammation, adipose tissue infiltration, hepatitis, etc.) are likely to become more common due to high-fat and increase in obesity. The link between the positive effects of probiotics and liver health is not direct, but seems to be based on prevention (Georgieva et al., 2015).

29.6.1.3 The radiation protection effect of probiotics

There are data on the protective effects of probiotics in chemotherapy and radiation. Radiation therapy is used in almost all types of tumors (cancer of the prostate, cervix, bladder, intestine, etc.). The side effects of it are very severe, which requires its cessation. The therapy kills both cancer and completely healthy cells. The mucosa is damaged throughout the digestive system, leading to severe nausea, vomiting, and diarrhea. Studies have shown that probiotics protect the lining of the digestive system from the harmful effects of radiation. An important condition is that probiotics be taken preventively and not after the onset of complications (Ciorba et al., 2011).

Probiotics, as living microbial supplements, help the body regain normal levels of "good" bacteria and boost immunity. There are about 500 different types of "good" bacteria. The two most common groups of probiotic bacteria are *Lactobacillus* and *Bifidobacterium*. Scientists have found that they protect against radiation damage. The mechanisms by which probiotics exert their radiation protection effect are discussed. Most likely it is due to their immune-stimulating and antioxidant effect. There are numerous studies on the effect of probiotic bacteria as antioxidants.

29.6.1.4 Antitumor and antimutagenic effect of probiotic bacteria—a problem with a wide social response

Cancer is one of the leading causes of death worldwide. They are considered to be diseases of economically developed and rich societies. The tendency is for these diseases to affect an increasing number of people. Colorectal cancer (CRC) is the second highest cancer mortality in the world and the most common localization of cancer in the digestive system. It is known that high levels of carcinogens are released in the GIT through diet. Obviously, diet, nutrition, and intestinal flora are key factors in the onset and development of colon cancer. Cancer therapy is a challenge that may not be impossible. The answer may lie in the effectiveness of some bacteria, known as probiotics or functional foods. One of the biggest challenges in modern science is to develop an effective methodology for inhibiting tumor growth. Chemotherapy and radiation therapy (Milenic et al., 2004; Van Cutsem et al., 2009) are by far the best ways to stop tumor growth, but they have many limitations (Dean et al., 2005; Knox & Meredith, 2000). In this context, finding an intrinsic way to inhibit tumor growth is one of the biggest challenges.

Probiotics can modulate the actions of the intestinal microflora through various mechanisms and thus show their anticancer activity. Probably, probiotics have different effects at different stages of carcinogenesis, which can be summarized as: antigenotoxicity, inhibition of enzyme activity in the colon, control of the growth of potentially harmful bacteria, interaction with colonocytes, stimulation of the immune system, and production of physiologically active metabolites. In general, the main mechanisms manifested by probiotic bacteria to cancer inhibition can be explained by a group of effects that depend on the type of probiotic strain. Ouwehand et al. (1999) described as early as 1999 the possible mechanisms of probiotics for cancer inhibition (Ouwehand et al., 1999).

In an experimental in vitro and in vivo study, Ma et al. (2008) showed that the probiotic *Bacillus polyfermenticus* inhibits the growth of colon cancer cells by reducing ErbB2 and ErbB3 receptors (which are known to play a significant role in tumor development) (Ma et al., 2008).

A modern study on the antitumor effects of *Lactobacillus Delbruckei* sp. *bulgaricus* strain DWT1 (LDB) and *Streptococcus thermophilus* strain DWT4 (ST), an established probiotic cocktail (LDB-ST), was performed on colon cancer cell lines by our team in 2015 (Georgiev et al., 2015). The probiotic cocktail showed promising primary screening results—on the one hand, low direct cytotoxicity and on the other, dose-dependent inhibition of proliferation on the colon cancer cell line—with HT-29 (in high concentration) (250−1000 μg/mL). (Fig. 29.2A and B).

In 2019, Palock and colleagues reported that the same probiotic formula containing *Lactobacillus bulgaricus DWT1* and ST could inhibit tumor growth by activating the proinflammatory response in macrophages (Guha et al., 2019). The

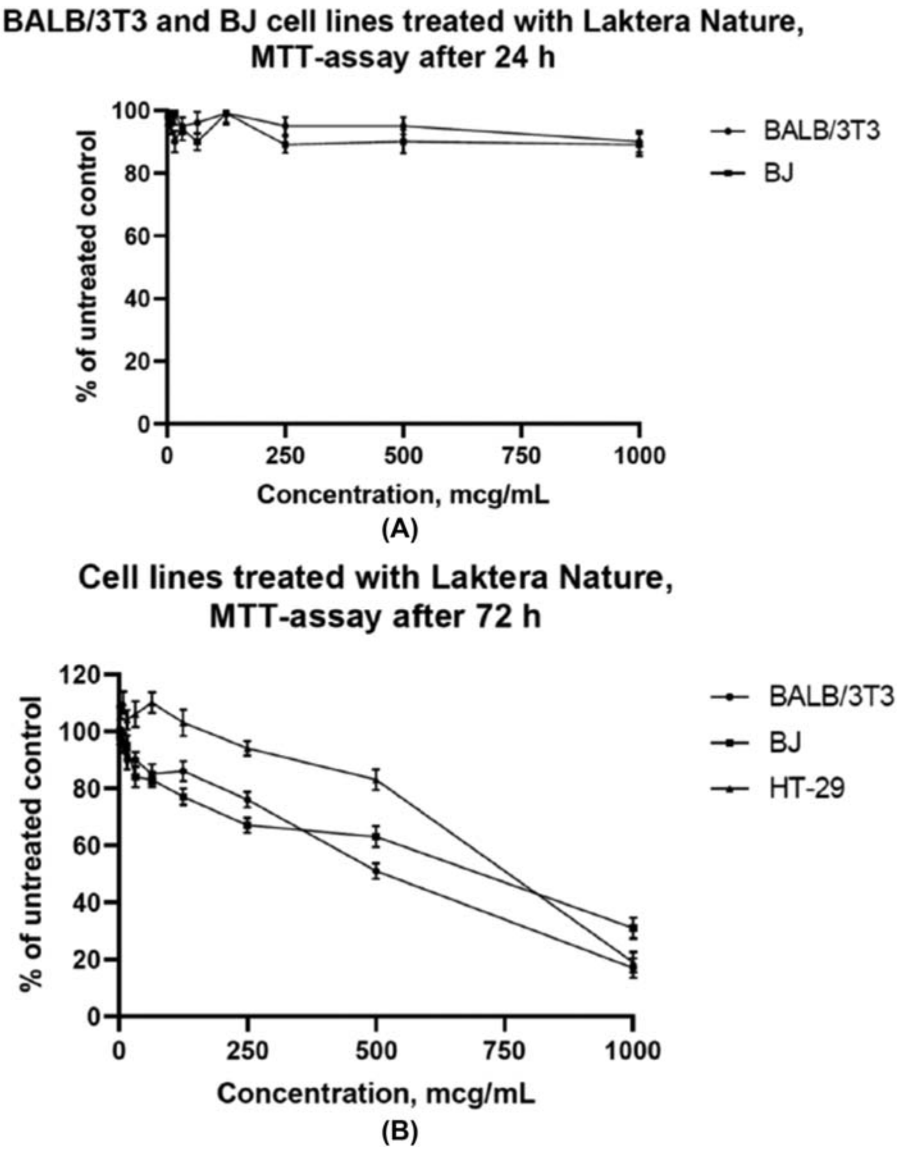

FIGURE 29.2 (A) BALB/3T3 and BJ cell lines treated with Laktera Nature in concentrations of 2–1000 μg/mL. MTT assay after 24 h. (B) Antiproliferative effects of probiotic food Laktera Nature used in concentrations of 2–1000 μg/mL on noncancerous (BALB/3T3 and BJ) and cancerous (HT-29, colon cancer) cell lines.

report revealed that after treatment with the established probiotic cocktail, the conversion of M2 to M1 state of macrophage cells of murine origin took place in vitro, ex vivo, and in vivo. The result of this study has the potential to develop newer strategies for inhibiting tumor growth.

29.7 Probiotics and metabolic syndrome

Metabolic syndrome is associated with an increased risk of cardiovascular disease and premature death. It is characterized by impaired glucose tolerance or type 2 diabetes, insulin resistance, as well as high blood pressure, dyslipidemia, decreased levels of high-density lipoprotein cholesterol (HDL-C), central obesity, and a body mass index $> 30\ kg/m^2$. Many factors are involved in the pathogenesis of the metabolic syndrome and they determine the individual approach for both prevention and treatment.

In the process of co-evolution, the HM has transformed from an accompanying commensal into a "metabolic organ." Genetic diversity in the microbial community provides a variety of enzymes and biochemical pathways, making the HM able to take on metabolic functions that complement human physiology. As the metabolic capacity of HM is equal to that of the liver, HM can be considered as an additional (virtual) organ in the GIT (Gill et al., 2006). The full metabolic potential of the microbiome has only recently been established and the potential contribution of HM to metabolic status and human health, as well as the link with obesity and related disorders, has been assessed (Jandhyala et al., 2015).

Probiotics are products of living microorganisms, mainly derived from *Lactobacillus* and *Bifidobacterium*. They have been the subject of research in recent years, related to their influence on the metabolic syndrome. Convincing facts show that probiotic bacteria improve digestion, absorption of nutrients, modulate the immune system, and lower elevated cholesterol. Probiotics are a possible future for patients with insulin resistance and obesity.

29.8 Probiotics and urogenital infections

It is believed that in the near future probiotics can largely replace conventional antibiotic therapy without side effects. There is a lot of scientific evidence for the role and application of *Lactobacilli* in maintaining vaginal microbial balance, prevention, and treatment of various urogenital infections in response to exhaustion of conventional therapy (Ding et al., 2017; Macklaim et al., 2015; Reid & Bruce, 2006).

29.9 Probiotics and immunity

Hippocrates (460−370 BCE) stated: "All diseases begin in the intestines." Both the diversity and the abundance of microorganisms in the gut play an important role in maintaining human health.

The stimulation of the immune system by probiotics and their connection with the "second brain"—the immune system in the gastrointestinal tract—has been established.

More than 80% of the cells of the immune system are located in the intestinal lymphoid tissue. The billions of cells of the immune system in the abdominal cavity form the "second brain" of the body of each individual. This "second brain" has the task of protecting and defending the body from bacterial aggression of pathogenic microorganisms. Our knowledge of this "second brain" and how it works is another challenge and is at the very beginning of our study. As knowledge progresses, we will create more and more perfect means to protect and stimulate the body's naturally built defense systems.

Neural pathways and central nervous system (CNS) signaling systems, according to new research, can be activated by bacteria in the gastrointestinal tract, including commensal, probiotic, and pathogenic bacteria. In theory, any disease associated with damage to the intestinal microbiota can be affected by its therapeutic modulation. Of particular interest are probiotic genes and probiotic factors involved in the regulation of host immunity, as well as the role and mechanisms of probiotics in disease prevention and treatment.

As you can see in Fig. 29.3, probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defense mechanisms, including innate and adaptive immune responses. The use of probiotics enhance the immune system by contributing with specific and nonspecific immune responses especially cytokines

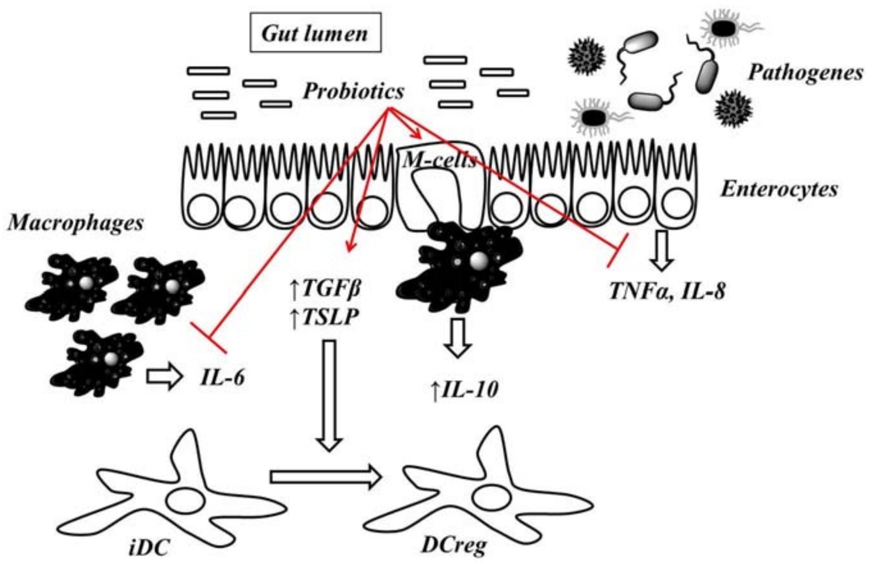

FIGURE 29.3 Probiotic modulation of the gastrointestinal mucosal immune system. While intestinal epithelial cells (IECs) exposed to pathogenic microbes or related stimuli produce proinflammatory mediators such as interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α), probiotics suppress the production of these cytokines and instead induce antiinflammatory mediators such as transforming growth factor β (TGF-β) and thymic stromal lymphopoietin (TSLP), which can promote the differentiation of immature dendritic cells (iDCs) to regulatory dendritic cells (DCregs). Macrophages in the inflamed mucosa produce high amounts of IL-6, and probiotics can decrease their IL-6 production and increase IL-10 production.

release, natural killer, macrophage activation, and increased immunoglobulin levels. This immune reactions occur without a noticeable harmful inflammatory response (Ashraf & Shah, 2014; Ding et al., 2017).

The human body and the intestinal microflora are in a symbiotic relationship, and the hypothesis of a "superorganism" composed of body and microbes has recently been proposed. The intestinal microbiota performs important metabolic and immunological tasks and damage to its composition can alter homeostasis and lead to the development of various diseases.

There are new discoveries related to the effect of probiotics on immunity, thanks to the rapid development of metagenomic strategies. This knowledge will support treatment focused on the specific effects of different probiotics and prebiotics on the intestinal microbiota.

29.10 Probiotics and mental illness called Plus Ultra

The challenge and growing interest is to establish the link between microbiotic dysbiosis and the occurrence and/or course of major mental disorders, and to explore potential therapeutic options for microbiotic dysbiosis in psychiatric patients.

Microbiotic dysbiosis can negatively affect the functioning of the CNS through various intertwined pathways that collectively form the "brain−gut" axis. The brain−gut axis includes the CNS, the neuroendocrine and neuroimmune systems, the autonomic nervous system, the enteric nervous system, and intestinal microbiota. Experimental studies suggest that the brain−gut axis may play an important role throughout life in regulating stress, anxiety, and mood. The intestinal microbiota is increasingly seen as a symbiotic partner in maintaining good health. Metagenomic approaches could help to understand how the complex intestinal microbial ecosystem is involved in controlling the development and functioning of the brain.

Plus Ultra reflects the expectation that probiotics will most likely be used regularly in the complex treatment of anxiety, depression, Alzheimer's disease, and autism. There are some prospects for future developments of therapeutic substances, such as probiotics, prebiotics, and nutritional approaches in the treatment of mental disorders.

The study of the role of the human intestinal microbiota in the genesis and/or maintenance of psychiatric disorders is in its infancy, but is currently one of the most promising directions for research in psychiatry (Park et al., 2018; Slykerman et al., 2017). Today, autism is a psychiatric disorder in which the role of the microbiota is best studied. Some therapeutic options aimed at regulating microbiotic dysbiosis have already been studied as probiotic use or dietary modifications, but with conflicting results (Kushak et al., 2011).

29.11 The next 45 years

Predicting the future has never been easy and is usually based on what we know today. But it seems clear that probiotics will be used to treat depression and anxiety and potentially other forms of mental illness (Kushak et al., 2011; Puebla-Barragan & Reid, 2019). Of course, studies are underway using fecal microbiota transplantation for multiple sclerosis, Parkinson's disease, and dementia, so that in the coming years the strains that may have an effect will be identified, tested, and will be part of medical practice for microbial intervention. (Park et al., 2018; Slykerman et al., 2017).

A deeper step would be to implant probiotic strains directly into the brain, perhaps to counteract pathogens or to produce certain chemicals, such as γ-aminobutyric acid or serotonin, in certain places to try to improve function. This will require the ability to manipulate strains and control their spread outside the implanted site—all within the capabilities of molecular genetics and biomedical engineering. Disclosure of ethical issues may take longer, depending on what metabolites are produced and what functions they affect (Puebla-Barragan & Reid, 2019).

No more difficult ethical issues will be raised than if probiotics affect the development of the fetal organs and its life course. This is certainly possible, given that the risk of Alzheimer's disease can be reduced if the mother is treated. Whether probiotics will be implanted in the uterus, or their functions will be passed on through the mother's metabolism, remains to be seen, but both can happen. Apparently, there are compounds, including neurochemicals, vitamins, lipids, and peptides produced by microbes that already affect the development and functioning of the body (Gong et al., 2019; Lyte & Brown, 2018; Patel et al., 2013; Robertson et al., 2017; Wang et al., 2018).

There is already preliminary evidence of improved cognitive function in adults (Ceccarelli et al., 2017).

29.12 Summary

For the past 45 years, probiotic microbes have been identified, tested, and administered to patients and users worldwide. The market of more than US$40 trillion reflects not only interest in natural therapies and a desire to avoid drugs that are often ineffective or with serious side effects, but also due to rigorous research (Global Market Insights, Inc., 2017). There may never be enough large, randomized placebo-controlled trials to satisfy all critics, but the number of lives saved and improved by probiotics continues to grow. As future technology advances, it will be seen that different types of probiotics will be applied in new ways to further improve the well-being of humans, as well as other hosts from fish to honeybees, livestock, and wildlife.

29.13 Probiotics and Covid-19: data supporting the use of probiotics to prevent Covid-19

Of great interest is the link between Covid-19, gut microbiota, lung immunity, and probiotics. Would probiotics help?

Despite strategies based on social distancing, hygiene, and screening, Covid-19 is advancing rapidly around the world, with the risk of overloading health systems. For the time being, the establishment of effective drug therapies continues, vaccines are not yet available, so additional preventive strategies are urgently needed. Clinical data indicate that some probiotic strains help prevent bacterial and viral infections, including gastroenteritis, sepsis, and respiratory infections (RTIs). The reason for adding probiotic strains to the overall prevention and care strategy is based on science and clinical trials, although so far there have been no direct studies on the etiological agent of this pandemic (Baud et al., 2020; Su et al., 2020).

French Professor Jean Bousquet has examined the relationship between the consumption of fermented vegetables and mortality from Covid-19 in various European countries in 2020. Some of the countries with low mortality from Covid-19 are those with relatively high consumption of traditional fermented foods. He concluded that the consumption of fermented vegetables significantly reduced Covid-19 mortality for these countries. For each gram per day increase in the average national consumption of fermented vegetables, the risk of death from Covid-19 decreased by 35.4% (95% CI: 11.4%, 35.5%).

Jean Bousquet found that Bulgaria, Greece, and Romania had very low mortality rates. This can also be linked to diet, as cabbage (Romania) and fermented milk (Bulgaria and Greece) are common (frequently consumed there) foods. These foods are a known natural inhibitors of angiotensin-converting enzyme (Bousquet et al., 2020).

Two meta-analyses of randomized controlled trials (RCTs) in humans have shown that probiotics can reduce the frequency and duration of respiratory viral infections.

In two RCTs, one with 146 and the other with 235 patients on mechanical ventilation, probiotic administration improved pneumonia outcomes compared with placebo (Gu et al., 2020).

Other researchers are bolder "Probiotics are usually safe, even in the most vulnerable populations and intensive care units," said Professor David Baud and colleagues in a review paper in the *Frontiers of Public Health*. "Instead of considering intensive care patients too ill to receive probiotic and prebiotic therapy, RCTs (randomized clinical trials) with probiotics for the prevention of ventilator-associated pneumonia give reason to keep them in mind" (Baud et al., 2020).

In addition, these researchers listed the appropriate probiotics to consider, during the Covid-19 pandemic.

Covid-19 is being presented with a spectrum of disease severity, ranging from mild and nonspecific flu-like symptoms to pneumonia and life-threatening complications such as acute respiratory distress syndrome (ARDS) and multiorgan failure. While transmission of SARS-CoV-2 is thought to occur primarily through respiratory droplets, the gut may also contribute to the pathogenesis of Covid-19 (Ng & Tilg, 2020). SARS-CoV-2 RNA was detected in gastrointestinal and fecal samples from patients (Pan et al., 2020; Jin et al., 2020; Lin et al., 2020) and in sewage systems (Wu et al., 2020). Coronaviruses, including SARS-Cov-2, can invade enterocytes, thus acting as a reservoir for the virus (Lin et al., 2020). In fact, large clinical studies from China have shown that gastrointestinal symptoms are common in Covid-19 and are related to the severity of the disease (Jin et al., 2020; Lin et al., 2020).

As reports from China indicate that Covid-19 may be associated with intestinal dysbiosis (causing inflammation) and a weaker response to pathogens (Gao et al., 2020; Xu et al., 2020), these cases will require restoration of homeostasis (Di Pierro, 2020). Oral administration of probiotic strains may further affect this gut-lung axis, as some of them may migrate from the gut to distant sites, e.g. in the breast for the treatment of mastitis (Arroyo et al., 2010).

Mechanisms that could explain the clinical success of probiotics include improving the intestinal epithelial barrier, competing with nutrient pathogens and adhesion to the intestinal epithelium, antimicrobial production, and modulation of the host immune system (Bermudez-Brito et al., 2012). The intestinal microbiome has a critical effect on systemic immune responses and immune responses in distant sites, including the lungs (Abt et al., 2012; Zelaya et al., 2016). The administration of some *Bifidobacteria* or *Lactobacilli* has a beneficial effect on the clearance of influenza virus from the respiratory tract (Ichinohe et al., 2011; Zelaya et al., 2016). The application of probiotic strains improve the levels of type I interferons, increase the number and activity of antigen-presenting cells, NK (natural killer) cells, and T cells, as well as the levels of systemic and mucosal specific antibodies in the lungs (de Vrese et al., 2005; Ichinohe et al., 2011; Zelaya et al., 2016). There is also evidence that probiotic strains modify the dynamic balance between proinflammatory and immunoregulatory cytokines that allow viral clearance while minimizing immune-mediated lung damage. This may be particularly important in preventing ARDS, a major complication of Covid-19. Evidence for antiviral activity of probiotic strains against common respiratory viruses, including influenza, rhinovirus, and respiratory syncytial virus, is the result of clinical and experimental studies (Luoto et al., 2014; Namba et al., 2010; Turner et al., 2017). Although none of these effects or mechanisms have been tested on the new SARS-CoV-2 virus, this approach should be considered, especially when beneficial effects of probiotics against other coronavirus strains have been reported (Chai et al., 2013; Kumar et al., 2010; Liu et al., 2020; Wang et al., 2019).

Since the link between viral replication and gastrointestinal immunity is very close, the effect of probiotic bacteria can play an important role in stopping viral replication. New approaches to single-strain probiotic bacteria can be promising in terms of both vaccination and treatment models (Bozkurt, 2020).

29.14 Conclusion

From the first description to today's knowledge of probiotics, the thread of knowledge continues to unravel. In today's global society, where people are exposed to daily stress, the widespread use of antibiotics, poor nutrition, and a number of harmful external factors, the balance of the gastrointestinal flora suffers from constant disturbances. And the ingenious conjecture of Hippocrates that "all diseases begin in the intestinal tract" finds its modern confirmation. It is no coincidence that in Europe, probiotics and probiotic foods are among the most stable segments of the food market.

However, it should be noted that probiotics, although harmless, are not a magic panacea. Continuous and adequate supervision of their use is necessary to prevent even the slightest risk to human health. This supervision should be exercised strictly by the relevant health institutions, because the maxim of Hippocrates: "Primum non nocere!" (First, do no harm!) remains in force to this day. Despite the proven health benefits, a probiotic per se is not able to prevent or treat the whole huge range of human diseases. But the thread of our knowledge must continue to unravel.

The ability to control and predict the effects of probiotics, based on rigorous research, would allow both consumers and manufacturers to choose and offer products with proven healing properties and thus fully extend human life.

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