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# Antimicrobial Peptides from Lactic Acid Bacteria

## Diversity, Biosynthesis and Applications

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*Dedicated to our teachers*

# Preface

In recent years, the global healthcare landscape has faced escalating challenges due to the rise of antibiotic resistance among pathogens. This crisis has sparked a pressing need for alternative strategies to combat microbial infections. Among the promising avenues of research, antimicrobial peptides (AMPs) derived from lactic acid bacteria have emerged as a fascinating area of study. These naturally occurring peptides possess potent antimicrobial properties, making them attractive candidates for therapeutic development.

The aim of this book, *Antimicrobial Peptides from Lactic Acid Bacteria*, is to provide a comprehensive overview of the latest research and advancements in this field. From exploring the diverse sources of lactic acid bacteria to elucidating the mechanisms of action of their AMPs, this volume delves into the intricate world of microbial defense mechanisms. Readers will find detailed discussions on the biosynthesis, purification, and characterization of AMPs from lactic acid bacteria, along with insights into their potential applications in various industries, including healthcare, food preservation, and agriculture. Additionally, the book examines the challenges and opportunities associated with harnessing these peptides for therapeutic purposes, paving the way for future breakthroughs in antimicrobial therapy. This book attempts to provide knowledge on the past, present, and future perspectives of lactic acid bacteria in diversified areas to cover a large group of readers and researchers interested in this field.

We are grateful to the contributors who have shared their expertise and insights to make this book possible. Their collective efforts have resulted in a valuable resource that will benefit researchers, healthcare professionals, and students interested in AMPs and lactic acid bacteria. Finally, we would like to acknowledge the support from the contributing authors and suggestions received from the editorial office at Springer, Emmy Lee, Lauren Kim, and Kamesh Senthilkumar. As editors, we hope that this book serves as a catalyst for further exploration and innovation in the field of AMP research. By fostering collaboration and knowledge exchange, we

aspire to contribute to the development of novel antimicrobial strategies that address the urgent global health threat posed by antibiotic resistance.

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# About the Editors

**Prasun Kumar, Ph.D.** holds a Ph.D. in Biotechnology from CSIR-Institute of Genomics and Integrative Biology, Delhi, India. He is presently working as an Assistant Professor, at Sharda University, Greater Noida, U.P., India. Earlier, he was working as an Assistant Professor at the Department of Chemical Engineering, Yeungnam University, Republic of Korea. He has over 7 years of experience in applied microbiological research including about 2 years of experience in industrial R&D. His main areas of research are biopolymers, microbial biodiversity, bioenergy, microbial biofilms, quorum sensing, quorum quenching, and genomics. His present research is oriented toward valorizing lignocellulosic biowastes into value-added products such as biopolymer, 2G ethanol, bioenergy, and antibiofilm compounds. To his credit, there are over 34 articles in SCI journals, 7 books, and 14 chapters with international publishers. He has been serving the scientific society by reviewing articles for several SCI journals and delivering guest lectures. Publons awarded him the peer review award in the year 2018. He also serves as the editorial board member of a few international journals.

**Subhasree Ray, Ph.D.** is currently working as an Assistant professor at Sharda University, Greater Noida, Uttar Pradesh, India. She earned her Ph.D. degree from CSIR-IGIB, Delhi in 2018. She received the prestigious CSIR-SRF fellowship. Her main research work was focused on the production of biopolymers from waste biomass. After Ph.D., she joined as a postdoctoral researcher at Ewha University and at the University of Seoul, South Korea. Here, her main focus was anaerobic digestion of food wastes for methane production. She also studied methanogenesis at 4000 L pilot-scale plant. After successful completion of 1 year, she joined another project at Yeungnam University, South Korea. During that period, she worked on several fungal toxins and their inhibition from fermented food. She also worked on biofilm inhibition of pathogenic organisms by natural bioactive compounds. To her credit, she has 24 research papers published in peer-reviewed journals, 8 book chapters, and 2 books. In addition, she is a life member of various scientific societies and also a member of various committees at the Sharda University for Graduate and Undergraduate programs.

**Manabendra Mandal, Ph.D.** is a Professor at the Department of Molecular Biology and Biotechnology, Tezpur University, Napaam, Sonitpur, Assam, India. He is actively working in the field of Microbial Biotechnology. His primary research interest is bioactive compounds for antibiofilm activity, biofuels and probiotics and nutrition. His group is actively involved in investigating natural products for antimicrobials, biodiesel and probiotic applications. In recent past, his lab has extensively investigated bioconversion of organic wastes and de-oiled algal residue into biodiesel and value-added products (carotenoids) using oleaginous microbes. His lab has received several government funds to conduct research projects in the related field. He has published more than 130 articles and several book chapters. He has guided 5 Ph.D. students and worked as Dean of Student Welfare. He is also an active member of various scientific societies such as Indian Science Congress Association and Association of Microbiologists of India. Dr. Mandal has also contributed substantially to the field as an active editor and reviewer for various SCI journals and serving the society by delivering guest lectures.

# Chapter 1
# Lactic Acid Bacteria: Taxonomy, Characteristic Features, Physiology, and Diversity

**G. R. Rama, F. Bucker, M. M. Salazar, Subhasree Ray, and Camille Eichelberger Granada**

**Abstract** The versatility of lactic acid bacteria (LAB) makes their use significant for the health and food industries. These bacteria belong to the phylum—Firmicutes, class—Bacilli, and order—Lactobacillales, and are known to convert sugars into lactic acid, thrive in low pH environments, and withstand challenging conditions. These characteristics contribute not only to their probiotic features, but also to their role in fermenting foods, where their metabolic capabilities, including acidification and bacteriocin production, aid in preservation and flavor development while inhibiting potential pathogens. With over 200 identified species spanning multiple genera, LAB exhibit diverse physiological traits and functional properties, making them a subject of interest for researchers and industries. This chapter provides an overview of LAB's taxonomy, characteristics, physiology, diversity, environmental adaptability, contributions to human health, and their crucial role in fermented foods production.

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## 1.1 Introduction

Lactic acid bacteria (LAB) stand out as a group of versatile and fascinating microorganisms. Known for their remarkable contributions to producing fermented foods and beverages, humans have used these bacteria for centuries. However, besides their relevance for the food industry, LAB have a rich diversity and a myriad of characteristics that extend their use to health-related products such as pharmaceuticals, nutraceuticals, cosmetics, and others (Dueñas and López 2022).

Taxonomically, these bacteria belong to the phylum—Firmicutes, class—Bacilli, and order—Lactobacillales. They are non-spore-forming diverse group of Gram-positive bacteria that convert sugars into lactic acid. This unique metabolic feature plays a central role in their physiology and contributes to numerous beneficial properties. A distinguishing feature of LAB is the ability to tolerate low pH environments. This characteristic makes them thrive in various habitats, including plants, animals, and fermented foods (Wang et al. 2021). In addition to their acid tolerance, LAB have other survival mechanisms that allow them to withstand challenging conditions, such as bile salts and antimicrobial substances. Such adaptive strategies contribute to their resilience and ability to colonize diverse ecological niches. This is the main reason for their probiotic features (Li and Han 2018).

Physiologically, LAB are intricately linked to their metabolic capabilities. Through the fermentation process, these bacteria convert sugars leading to acidification of their environment. This acidification plays a pivotal role in the preservation and flavor development of fermented foods providing a competitive advantage to LAB by inhibiting the growth of potential pathogens. Moreover, some LAB strains produce antimicrobial substances, known as bacteriocins, which further contribute to their ability to outcompete other microorganisms (Darbandi et al. 2022).

Lastly, the diversity of LAB is vast; there are over 200 species identified to date. The species encompass a wide array of genera, including *Lactobacillus* (recently divided into 23 new genera), *Lactococcus*, *Streptococcus*, *Enterococcus*, *Bifidobacterium*, *Leuconostoc*, and others (Holzapfel and Wood 2014). Each strain in the LAB group shows unique physiological traits, metabolic pathways, and functional properties, making them the subject of great interest of researchers and industries alike. In this chapter, we describe LAB's taxonomy, characteristic features, physiology, and diversity. In addition, we discuss their ability to survive in different environments, their contributions to human health and well-being, and their role in fermented food production.

## 1.2 Taxonomy of the Lactic Acid Bacteria Group

The LAB group was first defined by "Orla-Jensen" based only on phenotypic traits, such as optimal growth temperature, sugar use, and spectrum of metabolites produced (Orla-Jensen 1919). The author described bacteria that belong to *Aerococcus* spp., *Streptococcus* spp., *Pediococcus* spp., *Leuconostoc* spp., and *Lactobacillus* spp., which fit the general characteristics of the group. In 1957, Bergey's Manual included *Bifidobacterium* spp. in this group, even though this genus was genetically similar to the actinomycete group. Then, a taxonomic revision divided the *Streptococcus* genus into three separate genera: *Lactococcus*, *Enterococcus*, and *Streptococcus*. A group that belonged to *Lactobacillus* spp. was named *Carnobacterium* spp. (Collins et al. 1987). Some motile bacteria from *Lactococcus* spp. were reclassified as *Vagococcus* spp. (Collins et al. 1989). *Pediococcus halophilus* formed a new genus, *Tetragenococcus* (Collins et al. 1990). Heterofermentative bacterial strains belonging to *Lactobacillus* spp. and *Leuconostoc* spp. were, then, separated in a new genus, *Weissella* (Collins et al. 1993).

New studies on molecular biology resulted in several changes in the taxonomy of the LAB group. These changes made LAB classification very confusing because 16S rRNA sequences are insufficient to propose phylogenetic relationships among LAB (Holzapfel and Wood 2014). Nevertheless, several authors did it, and LAB taxonomy was considered a "storm" (Qiao et al. 2022). Holzapfel and Wood (Holzapfel and Wood 2014) proposed 14 LAB genera: *Oenococcus*, *Pediococcus*, *Alloiococcus*, *Vagococcus*, *Aerococcus*, *Carnobacterium*, *Streptococcus*, *Lactobacillus* spp., *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Tetragenococcus*, *Weissella*, and *Bifidobacterium*. However, based on genetic advances, over 300 species of LAB were reorganized that belonged to seven genera and two families into a single *Lactobacillaceae* family (Zheng et al. 2020). The authors also divided the *Lactobacillus* genus into 25 different genera, 23 of which were new (Table 1.1). The LAB group is composed by 25 new lactobacilli and the remaining 13 genera (Holzapfel and Wood 2014). Lactobacilli, *Leuconostoc*, *Pediococcus*, *Lactococcus,* and *Streptococcus* are recognized as the main genera in the LAB group. However, all LAB present biotechnological potential for developing special foods and promoting health. In the next section, we discuss the most important features of LAB and their physiology.

## 1.3 Characteristic Features and Physiology

LAB belong to an extensive group of cocci or bacilli, Gram-positive, non-endospore-forming, catalase and oxidase-negative bacteria with strong tolerance to low pH. They are found in diverse habitats. The genera in this group also differ regarding tolerance to salt, optimum growth temperature, habitats, and pathogenicity. Most studied LAB strains are common in foods (dairy products, meat, vegetables,

**Table 1.1** Lactobacilli genera and species

| New genera | Species composition |
|---|---|
| ***Lactobacillus*** | *L. acetotolerans, L. acidophilus, L. amylolyticus, L. amylovorus, L. apis, L. bombicola, L. colini, L. crispatus, L. delbrueckii, L. equicursoris, L. fornicalis, L. gallinarum, L. gasseri, L. gigeriorum, L. hamster, L. helsingborgensis, L. helveticus, L. hominis, L. iners, L. intestinalis, L. jensenii, L. johnsonii, L. kalixensis, L. kefiranofaciens, L. kimbladii, L. kitasatonis, L. kullabergensis, L. melliventris, L. mulieris, L. panisapium, L. paragasseri, L. pasteurii, L. porci, L. psittaci, L. rodentium, L. taiwanensis, L. ultunensis, L. xujianguonis* |
| ***Amylolactobacillus*** | *A. amylophilus, A. amylotrophicus* |
| ***Holzapfeliella*** | *Holzapfeliella floricola* |
| ***Bombilactobacillus*** | *B. apium, B. bombi, B. folatiphilus, B. mellifer, B. mellis, B. thymidiniphilus* |
| ***Companilactobacillus*** | *C. allii, C. alimentarius, C. baiquanensis, C. bobalius, C. crustorum, C. farciminis, C. formosensis, C. furfuricola, C. futsaii, C. ginsenosidimutans, C. halodurans, C. heilongjiangensis, C. huachuanensis, C. hulinensis, C. insicii, C. jidongensis, C. kedongensis, C. keshanensis, C. kimchiensis, C. kimchi, C. metriopterae, C. mindensis, C. mishanensis, C. musae, C. nantensis, C. nodensis, C. nuruki, C. paralimentarius, C. salsicarnum, C. suantsaicola, C. tucceti, C. versmoldensis, C. zhachilii, C. zhongbaensis* |
| ***Lapidilactobacillus*** | *L. achengensis, L. bayanensis, L. concavus, L. dextrinicus, L. gannanensis, L. luobeiensis, L. mulanensis, L. wuchangensis* |
| ***Agrilactobacillus*** | *A. composti, A. fermenti, A. yilanensis* |
| ***Schleiferilactobacillus*** | *S. harbinensis, S. perolens, S. Shenzhenensis* |
| ***Lacticaseibacillus*** | *L. absianus, L. baoqingensis, L. brantae, L. camelliae, L. casei, L. chiayiensis, L. daqingensis, L. hegangensis, L. hulanensis, L. jixianensis, L. kribbianus, L. manihotivorans, L. mingshuiensis, L. nasuensis, L. pantheris, L. paracasei, L. parakribbianus, L. porcinae, L. rhamnosus, L. saniviri, L. sharpeae, L. songhuajiangensis, L. suibinensis. L. suilingensis, L. thailandensis, L. yichunensis, L. zeae, L. zhaodongensis* |
| ***Paralactobacillus*** | *Paralactobacillus selangorensis* |
| ***Latilactobacillus*** | *L. curvatus, L. fragifolii, L. fuchuensis, L. graminis, L. sakei* |
| ***Loigolactobacillus*** | *L. backii, L. bifermentans, L. binensis, L. coryniformis, L. iwatensis, L. jiayinensis, L. rennini, L. zhaoyuanensis* |
| ***Dellaglioa*** | *Dellaglioa algida* |
| ***Liquorilactobacillus*** | *L. aquaticus, L. cacaonum, L. capillatus, L. ghanensis, L. hordei, L. mali, L. nagelii, L. oeni, L. satsumensis, L. sicerae, L. sucicola, L. uvarum, L. vini* |
| ***Ligilactobacillus*** | *L. acidipiscis, L. agilis, L. animalis, L. apodeme, L. araffinosus, L. aviaries, L. ceti, L. equi, L. faecis, L. hayakitensis, L. murinus, L. pabuli, L. pobuzihii, L. ruminis, L. saerimneri, L. salitolerans, L. salivarius, L. ubinensis* |
| ***Lactiplantibacillus*** | *L. argentoratensis, L. daoliensis, L. daowaiensis, L. dongliensis, L. fabifermentans, L. garii, L. herbarum, L. modestisalitolerans, L. mudanjiangensis, L. nangangensis, L. paraplantarum, L. pentosus, L. pingfangensis, L. plajomi, L. plantarum, L. songbeiensis, L. xiangfangensis* |

(continued)

**Table 1.1** (continued)

| New genera | Species composition |
|---|---|
| ***Furfurilactobacillus*** | *F. curtus, F. milii, F. rossiae, F. iliginis* |
| ***Paucilactobacillus*** | *P. hokkaidonensis, P. kaifaensis, P. nenjiangensis, P. oligofermentans, P. suebicus, P. vaccinostercus, P. wasatchensis* |
| ***Limosilactobacillus*** | *L. agrestis, L. albertensis, L. alvi, L. antri, L. balticus, L. caviae, L. coleohominis, L. equigenerosi, L. fastidiosus, L. fermentum, L. frumenti, L. gastricus, L. gorillae, L. ingluviei, L. mucosae, L. oris, L. panis, L. pontis, L. portuensis, L. reuteri, L. rudii, L. secaliphilus, L. urinaemulieris, L. vaginalis* |
| ***Secundilactobacillus*** | *S. angelensis, S. collinoides, S. folii, S. hailunensis, S. kimchicus, S. malefermentans, S. mixtipabuli, S. odoratitofui, S. oryzae, S. paracollinoides, S. pentosiphilus, S. silagei, S. silagincola, S. similis, S. yichangensis* |
| ***Fructilactobacillus*** | *F. carniphilus, F. cliffordii, F. florum, F. fructivorans, F. hinvesii, F. ixorae, F. lindneri, F. myrtifloralis, F. sanfranciscensis, F. vespulae* |
| ***Acetilactobacillus*** | *Acetilactobacillus jinshanensis* |
| ***Apilactobacillus*** | *A. apinorum, A. apisilvae, A. bombintestini, A.kunkeei, A. micheneri, A. nanyangensis, A. ozensis, A. quenuiae, A. timberlakei, A. xinyiensis, A. zhangqiuensis* |
| ***Lentilactobacillus*** | *L. buchneri, L. curieae, L. diolivorans, L. farraginis, L. fungorum, L. hilgardii, L. kefiri, L. kisonensis, L. kosonis, L. kribbianus, L. laojiaonis, L. otakiensis, L. parabuchneri, L. parafarraginis, L. parakefiri, L. raoultii, L. rapi, L. senioris, L. sunkii* |
| ***Levilactobacillus*** | *L. acidifarinae, L. andaensis, L. angrenensis, L. bambusae, L. brevis, L. cerevisiae, L. enshiensis, L. fujinensis, L. fuyuanensis, L. hammesii, L. huananensis, L. humaensis, L. koreensis, L. lanxiensis, L. lindianensis, L. mulengensis, L. namurensis, L. parabrevis, L. paucivorans, L. senmaizukei, L. spicheri, L. suantsaii, L. suantsaiihabitans, L. tangyuanensis, L. tongjiangensis, L. wangkuiensis, L. yonginensis, L. zymae* |

Lactobacilli genera division developed by Zheng et al. (2020) and species composition adapted from LPSN (available in https://www.bacterio.net/)

among others), plants, and the intestinal tracts of humans and animals. They are fastidious microorganisms and require a broad range of complex nutrients for their survival and growth (Pessione 2012; Holzapfel and Wood 2014).

In addition, LAB do not carry out the oxidative phosphorylation pathway due to lack of cytochromes and porphyrins. These bacteria obtain energy (ATP generation) only through substrate-level phosphorylation. They are not sensitive to $O_2$, thus considered "aerotolerant anaerobes." They have an exclusively fermentative metabolism, with lactic acid ($D_{(-)}$ and/or $L_{(+)}$ lactic acid) as major end product. Nonetheless, LAB are also recognized for their metabolic capacity to produce a several biomolecules with different industrial applications, such as flavoring and antioxidant substances, vitamins, bacteriocins, and exopolysaccharides (EPS). The main compounds degraded by LAB are sugars. However, some LAB have the

ability to degrade proteins and mycotoxins, among a variety of other molecules (Wang et al. 2021).

According to the final fermentation products of glucose, LAB (except *Bifidobacterium* spp.) may be categorized into two groups: heterofermentative and homofermentative. In the latter case, lactic acid is the only final product of fermentation since hexoses are almost completely converted into lactic acid through glycolysis via the Embden–Meyerhof–Parnas (EMP) pathway. Two units of lactic acid are formed for every glucose molecule. Fructose-1,6-diphosphatase is the main enzyme of this pathway. Heterofermentative LAB can produce ethanol and $CO_2$ (one unit of each for every molecule of glucose) besides lactic acid. In this case, hexose sugars get fermented via the phosphoketolase (6-phosphogluconate) pathway, in which phosphoketolase is the main catalyst.

The type of hexose fermentation pathway used is mainly defined in LAB families. Among the 38 LAB genera previously discussed, only *Bifidobacterium* spp. do not belong to *Lactobacillales*. This order comprises five families: *Streptococcaceae*, *Enterococcaceae*, *Lactobacillaceae*, *Aerococcaceae*, and *Carnobacteriaceae*. The first two and most *Lactobacillaceae* are homofermentative (Holzapfel and Wood 2014; Zheng et al. 2020).

Also, LAB classification according to carbohydrate metabolism considers LAB ability to ferment pentoses, e.g., xylose, arabinose, ribose, and related compounds such as glucuronate. Homofermentative LAB may be divided into (i) obligate homofermentative, (ii) obligate heterofermentative, or (iii) facultative heterofermentative. In (i), only hexoses are fermented through the EMP pathway while pentose and related compounds are not converted. There are no phosphoketolase enzymes in this group, which includes strains of *Pediococcus*, *Lactococcus*, *Streptococcus* spp., and *Lactobacillus* spp. (*Amylolactobacillus*, *Holzapfeliella*, *Paralactobacillus*, among others). In (ii), LAB strains can ferment pentoses, glucose, and other related compounds via phosphoketolase pathway. This group lacks the fructose 1,6-diphosphate enzyme. Lastly, in (iii), there are genera, such as *Leuconostoc* and a few *Lactobacillus* spp. (such as *Schleiferilactobacillus*), that convert hexoses into lactic acid through glycolysis. However, they can also convert pentoses and related compounds via phosphoketolase pathway since they present both fructose 1,6-diphosphate and phosphoketolase enzymes (Pessione 2012; Holzapfel and Wood 2014; Zheng et al. 2020).

In terms of hexoses, most of these carbohydrates are fermented by LAB, e.g., fructose, mannose, and galactose. The first two can be fermented via EMP or phosphoketolase pathway. However, galactose can be fermented by different pathways depending on the mechanism by which sugar is transported across the cell membrane (Holzapfel and Wood 2014). Regarding pentoses, most heterofermentative LAB can ferment these sugars, although some strains are classified as pentose-negative. Pentoses are generally transported across the membrane by specific permeases and converted into D-xylulose 5-phosphate to be fermented by the phosphoketolase pathway (Kandler 1983). Lastly, some LAB strains can ferment disaccharides such as sucrose, lactose, cellobiose, and maltose. These

carbon sources are generally transported (across the membrane) by permeases or mediated by phosphorylation. Then, they are hydrolyzed into monosaccharides or one monosaccharide-phosphate and one monosaccharide, and fermented via EMP or phosphoketolase pathway depending on the strain (Yu et al. 2020).

## 1.4 Characteristics of the Most Studied LAB

There are several works describing characteristics and biotechnological potentials of all genera of LAB. Here, we described the most known LAB: the Lactobacilli group, *Lactococcus* app., *Enterococcus* spp., *Streptococcus* spp., and *Bifidobacterium* spp.

### *1.4.1 Lactobacilli*

Due to a recent reclassification of the *Lactobacillus* genus, the term lactobacilli is used here to refer to all emended genera *Lactobacillus, Paralactobacillus*, and the 23 new genera previously cited. Cells of this group are rod-shaped with facultative anaerobic metabolism. Besides lactate, other end products of lactobacilli's metabolism are acetate, ethanol, $CO_2$, formic acid, or succinate. In the order Lactobacillales, the *Lactobacillaceae* family is the only one that includes homofermentative and heterofermentative microorganisms, exemplifying the diversity of this group. However, most strains cannot ferment pentoses, and none of these bacteria encode genes for the pentose–phosphate pathway or pyruvate formic acid lyase. The ability to ferment different sugars remains strain-specific.

### *1.4.2 Lactococcus*

Lactococci are coccoid cells, non-motile that can occur individually, in pairs, or in short chains. Lactococci are generally found on plants, animals' skins, and dairy products. They are facultatively anaerobic and mesophilic, growing at a temperature of 10 °C but not at 45 °C. *Lactococcus* spp. produce only L-(+)-lactic acid via the EMP pathway. However, given specific conditions, some strains can produce different metabolites such as acetate, formic acid or $CO_2$, and ethanol (Garrigues et al. 1997). In general, lactococci strains can ferment hexoses and pentoses (Naranjo et al. 2022). Interestingly, their lactose metabolism differs from those of other LAB species since these bacteria can ferment galactose and glucose simultaneously. The latter is metabolized through tagatose pathway. Lastly, *Lactococcus* species can also metabolize proteins, fats, and citric acid, resulting in the production of volatile aroma and flavor compounds (Holzapfel and Wood 2014).

### 1.4.3 *Enterococcus*

Enterococci are non-motile (except for two species), ovoid cocci that can occur in single, pairs, and short chains. *Enterococcus* spp. can ferment a variety of carbohydrates, including hexoses, pentoses, and carbohydrate polymers, since they are facultatively heterofermentative with the addition of other molecules such as glycerol and citrate. An interesting feature of enterococci is that they are highly tolerant to desiccation, extreme pH values, osmotic and oxidative stresses, and metal concentrations. They also have resistance to several antimicrobial molecules. In general, the optimal growth occurs at 37 °C, but many strains may propagate within a range of 10–45 °C. This versatility enables enterococci to colonize in competitive environments, as many species of this genus are found in soils and as commensal bacteria in the gastrointestinal tracts (GIT) of insects, birds, reptiles, and mammals (Holzapfel and Wood 2014).

### 1.4.4 *Streptococcus*

Streptococci are non-motile cocci (ovoid or spherical shape) that occur mostly in chains or pairs. They grow under facultatively aerobic conditions. Some need $CO_2$ for growth. They require an optimum temperature for growth, i.e., 37 °C, but there is variation among strains. Streptococci are homofermentative and produce $L_{(+)}$ lactic acid as the key final product of glucose-fermentation via the glycolysis (EMP) pathway (Delorme 2008; Holzapfel and Wood 2014).

Most streptococci are facultative anaerobic, mostly opportunistic pathogenic, and belonging to human and/or animals commensal microbiota. Among streptococci species found in food, only *Streptococcus thermophilus* is having biotechnological potential in the dairy industry (Delorme 2008). *S. thermophilus* is a thermophilic bacterium (grows in temperatures up to 45 °C) and metabolizes other carbohydrates but prefers lactose. Lactose is transported through permease system followed by β-galactosidase hydrolysis releasing galactose and glucose. Glucose, on the one hand, is then fermented into lactic acid. Few secondary metabolites such as acetaldehyde, ethanol, acetate, and diacetyl may also be formed. Conversely, most *S. thermophilus* strains do not metabolize galactose, and the monosaccharide is secreted to the environment (Delorme 2008; Yu et al. 2020).

### 1.4.5 *Bifidobacterium*

Bifidobacterium is the only genus among LAB that are not included in the Firmicute phylum; they belong to Actinobacteria. They are Gram positive, non-spore-forming, non-motile, anaerobic bacteria with a Y-shape or a “bifid” morphology.

Bifidobacteria are mostly found in the GIT of animals and humans. They can also be found in sewage, milk, oral cavity, human blood, and hindgut of social insects and birds. An important feature of this genus is its monosaccharide metabolism. Bifidobacteria use a singular route of fructose 6-phosphate pathway for monosaccharide degradation or "bifidus pathway," which produces 1 mol lactic acid /mol glucose and 1.5 molecule of acetic acid. Fructose 6-phosphate phosphoketolase (*Xfp*) is the main enzyme of this pathway, and its activity is a common phenotypic test for differentiating bifidobacteria since this enzyme is absent in other Gram-positive GIT microorganisms. Lastly, it is worth mentioning that *Bifidobacterium* spp. have developed adaptive metabolic strategies to survive hostile conditions of the upper parts of the intestine. They can metabolize an array of complex host- and diet-derived glycans while producing an arsenal of proteins and enzymes to fight environmental stresses (Holzapfel and Wood 2014; Alessandri et al. 2021). Bifidobacteria are widely known for their probiotic features, which will be discussed in the next section.

## 1.5 Biotechnological Applications of LAB

Lactic acid bacteria have been used for various applications in the industry, both as health promoters and food additives. Regarding the improvement of human health, the most commonly reported feature of LAB is their probiotic function, providing the host with health benefits when administered in suitable amounts (Hill et al. 2014). As food additives, LAB are more commonly used as either non-starter or starter cultures, that is, as acid or aroma producers, respectively. This section discusses both biotechnological applications, and a summary of them is depicted in Fig. 1.1.

### *1.5.1 Applications of Lactic Acid Bacteria in Health Industry*

Well-designed studies, such as double-blind randomized controlled trials with placebo groups, evaluating the benefits of the administration of LAB in humans, are scarce. However, a recent review has retrieved 95 articles written in English after a search in the Scopus database for the words "clinical trial" OR "intervention" OR "treatment" AND "probiotic" OR "lactic acid bacteria." Of those 95 articles, 57 reported positive health outcomes, meaning they achieved an improvement or resolved the issue approached in the study (De Filippis et al. 2020). Of those 57, eight did not include a placebo group or did not explain the study design. These studies were excluded from this analysis. Hence, Table 1.2 lists all 49 studies reporting the positive outcomes of the use of probiotics for human health.

In Table 1.2, the 49 studies were allocated into five groups according to the target health treatment: the use of probiotic LAB for treating GIT-related issues counts the

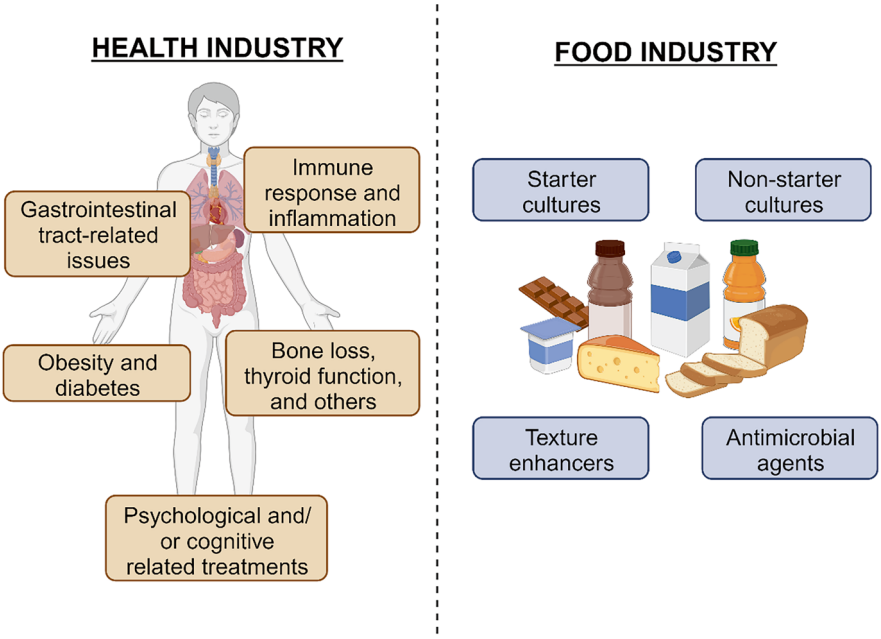


**Fig. 1.1** Biotechnological applications of lactic acid bacteria in both health and food industries

highest number of clinical trials, allocating 21 articles; following that, 10 studies mentioned probiotics for the treatment of obesity and diabetes-related issues; eight articles addressed psychological features and/or cognitive-related treatments; and seven studies reported probiotics as immune response enhancers and treatment for inflammation. Additionally, three studies mentioned different target treatments and were therefore allocated in the group called "Others": treatment for thyroid disfunctions, for hepatic encephalopathy in patients with cirrhosis, and for bone loss in older women. Given that these topics are uncommon, they will not be further discussed in this section. The other groups are discussed below.

#### 1.5.1.1 Probiotics as a Treatment for GIT-Associated Issues

Of all health targets listed in Table 1.2, probiotic LAB are commonly associated with the treatment of GIT-related diseases, with 21 out of the 47 articles listed in Table 1.2. One of the mostly mentioned topic is the treatment of symptoms of irritable bowel syndrome (IBS). IBS is a GIT disorder affecting up to 10% of otherwise healthy subjects who may experience symptoms such as abdominal pain and irregular bowel habits (Ford et al. 2020). For example, Cremon et al. (2018) report evidence that the microorganisms that colonize GIT are an important part of IBS pathophysiology. The authors found a shift in the GIT microbiota, identifying that the consumption of *Lb. paracasei* CNCM I-1572 can reduce the relative abundance

**Table 1.2** Clinical trials on the use of probiotics with positive health outcomes (adapted from De Filippis et al. (2020))

| Treatment for | Probiotic species/strains | Treatment | Target health outcome | Reference |
|---|---|---|---|---|
| GIT-related issues | *Lb. plantarum* KCTC 10782BP, DUOLAC 7—*S. thermophilus* KCTC 11870BP, *Lb. acidophilus* KCTC 11906BP, *B. lactis* KCTC 11904BP, *Lb. rhamnosus* KCTC 12202BP, *B. longum* KCTC 12200BP, Enterolactis® Plus—*Lb. paracasei* DG *B. brevis* KCTC 12201BP | 2 capsules/day, $5 \times 10^9$ CFU/capsule | Endotoxemia in obese subjects | Lee et al. (2014) |
| | | Not reported | IBS symptoms in adults | Cremon et al. (2018) |
| | Heat-killed *Lb. paracasei* CBA L74 | 1 serving/day, $5.9 \times 10^{11}$ CFU/serving | Prevention of infections in children (24–48 months) | Corsello et al. (2017) |
| | LacClean Gold-S®—*B. bifidum* KCTC 12199BP, *B. lactis B. longum* KCTC 12200BP, KCTC 11904BP, *Lb. acidophilus* KCTC 11906BP, *S. thermophilus* KCTC 11870BP, *Lb. rhamnosus* KCTC 12202BP, | 2 capsules/day, $5 \times 10^9$ CFU/capsule | IBS symptoms in adults | Yoon et al. (2014) |
| | *Lb. acidophilus* PBS066, *Lb. plantarum* PBS067 OR *Lb. reuteri* PBS072, *Lb. rhamnosus* LRH020, *B. animalis subsp. lactis* BL050, | 1 serving/day, $5 \times 10^9$ CFU/serving | IBS with constipation in adults | Mezzasalma et al. (2016) |
| | *Lb. acidophilus*, *B. longum*, strains not reported | 2 capsules/day, counts not reported | IBS symptoms in adults | Cui and Hu (2012) |
| | *Lb. casei* LMG 101/37 P-17 504, *Lb. plantarum* CECT 4528, *B. animalis* subsp. *lactis* Bi1 LMG P-17 502, *B. breve* Bbr8 LMG P-17 501, *B. breve* Bl10 LMG P-17 500 | 1 sachet/day, $1 \times 10^9$ CFU/sachet | Celiac disease in adults with IBS | Francavilla et al. (2019) |
| | *Lb. casei*, *Lb. rhamnosus*, *S. thermophilus*, *B. breve*, *Lb. acidophilus*, *B. infantis*, *Lb. delbrueckii* subsp. *bulgaricus*; strains not reported | 1 capsule/day, $1.5 \times 10^{10}$ CFU/capsule | Crying time in infants with colics | Kianifar et al. (2014) |
| | *Lb. coryniformis* CECT5711, *Lb. gasseri* CECT5714 | 1 serving/day, $2 \times 10^9$ CFU/serving | Bowel habits in adults | Olivares et al. (2006) |
| | *Lb. plantarum* ATCC 202 195 | 1 capsule/day, $1 \times 10^9$ CFU/capsule | Neonatal sepsis | Panigrahi et al. (2017) |

(continued)

**Table 1.2** (continued)

| Treatment for | Probiotic species/strains | Treatment | Target health outcome | Reference |
|---|---|---|---|---|
| | *Lb. reuteri* DSM 17 938 | 5 drops/day, $0.2 \times 10^8$ CFU/drop | Crying time in infants with colic | Savino et al. (2010) |
| | *Lb. reuteri* DSM 17 938 | 5 drops/day, $0.2 \times 10^8$ CFU/drop | Crying time in infants with colic | Savino et al. (2018) |
| | *Lb. rhamnosus* GG | 1 serving/day, $6 \times 10^9$ CFU/serving | Intestinal inflammation in children with cystic fibrosis | Bruzzese et al. (2014) |
| | *Lb. rhamnosus* GG, *Lb. rhamnosus* LC705, *Propionibacterium freudenreichii* subsp. *shermanii* JS, *B. animalis* subsp. *lactis* BB12 | 120 mL/day, $5 \times 10^9$ CFU/mL | IBS symptoms in adults | Kajander et al. (2007) |
| | *Lb. rhamnosus* IMC 501, *Lb. paracasei* IMC 502 | 1 serving/day, $1 \times 10^9$ CFU/serving | Bowel habits in adults | Verdenelli et al. (2011) |
| | Symprove—*Lb. rhamnosus* NCIMB 30 174, *Lb. plantarum* NCIMB 30 173, *Lb. acidophilus* NCIMB 30 175, *Enterococcus faecium* NCIMB 30 176 | 1 serving/day, $1 \times 10^{10}$ CFU/serving | IBS symptoms in adults | Sisson et al. (2014) |
| | VSL#3®—*S. thermophilus*, *B. breve, B. longum, B. infantis, Lb. acidophilus, Lb. plantarum, Lb. paracasei, Lb. delbrueckii;* strains not reported | 1 capsule/day, $1.1 \times 10^{11}$ CFU/capsule | Diarrhea-predominant IBS symptoms in adults | Michail and Kenche (2011) |
| | VSL#3® (same as above) | 2 sachets/day, $9 \times 10^{11}$ CFU/sachet | IBS symptoms in adults | Ng et al. (2013) |
| | Yakult®—*Lb. casei* Shirota | 1 bottle/day, $1 \times 10^{11}$ CFU/bottle | Stress-induced abdominal dysfunction in adults | Kato-Kataoka et al. (2016) |
| | Zircombi—*B. longum* BB536, *Lb. rhamnosus* HN001 | 1 sachet/day, $4 \times 10^9$ CFU/sachet | Symptoms of lactose intolerance in adults | Vitellio et al. (2019) |
| | *Lb. johnsonii* LA1 | 80 mL/day, $1 \times 10^7$ CFU/mL | Eradication of infection by *Helicobacter pylori* in children | Gotteland et al. (2008) |