REVIEW ARTICLE

The food-gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health

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ABSTRACT

Lactic acid bacteria (LAB) are present in foods, the environment and the animal gut, although fermented foods (FFs) are recognized as the primary niche of LAB activity. Several LAB strains have been studied for their health-promoting properties and are employed as probiotics. FFs are recognized for their potential beneficial effects, which we review in this article. They are also an important source of LAB, which are ingested daily upon FF consumption. In this review, we describe the diversity of LAB and their occurrence in food as well as the gut microbiome. We discuss the opportunities to study LAB diversity and functional properties by considering the availability of both genomic and metagenomic data in public repositories, as well as the different latest computational tools for data analysis. In addition, we discuss the role of LAB as potential probiotics by reporting the prevalence of key genomic features in public genomes and by surveying the outcomes of LAB use in clinical trials involving human subjects. Finally, we highlight the need for further studies aimed at improving our knowledge of the link between LAB-fermented foods and the human gut from the perspective of health promotion.

Keywords: food microbiome; human microbiome; lactic acid bacteria; probiotics

INTRODUCTION

The lactic acid bacteria (LAB) group is phylogenetically located in the Clostridia branch of Gram-positive bacteria and includes non-spore-forming cocci, coccobacilli or rods, and aerobic-tolerant anaerobes, with a molar DNA base composition of less than 50% G + C (Pot et al. 1994). LAB are among the most widely studied microorganisms worldwide. Given the important role that LAB play in different biotechnological processes, it is not surprising that they have received much attention from the scientific community for decades. A search for the term ‘lactic acid bacteria’ in the title, keywords and abstract in the scientific database Scopus (www.scopus.com) (Burnham 2006) returned approximately 32,700 documents at the time of this review (May 2020). In addition, using ‘lactic acid bacteria’ AND ‘food’, ‘lactic acid bacteria’ AND ‘gut’ or ‘lactic acid bacteria’ AND ‘environment’ as search terms, 11,800, 1,500 and 1,700 documents can be retrieved, respectively, which clearly indicates that food is the most widely studied environment in association with LAB.

Although LAB exhibit considerable species and strain diversity and can play a significant role in different ecosystems, food remains their major source and preferred activity niche. This is mainly because the fermentation activity of LAB has been associated with foods and studied in fermented foods (FFs) since early 1900s. LAB activity in FFs can be basically considered a transformation of raw materials to edible food products with different characteristics. Food fermentation is actually an...
ancient process that was used as a strategy for food preservation, dating back to 10,000 years ago when agriculture and farming were introduced (Cordain et al. 2005). Food fermentation can be aerobic, such as alkaline fungal fermentation, and anaerobic, such as alcoholic and lactic acid fermentation by yeast and LAB, respectively (Nout 2014).

Some LAB strains are also considered potential probiotics, and many are commercialized in probiotic preparations and/or functional foods. In addition, they are also members of the gut microbiome of human and animal hosts, although their origin, role and potential activities are still widely discussed.

In this review, we discuss the occurrence of LAB species in both food and the human gut. Moreover, we assess the availability and information retrievable from available genomic and metagenomic data for LAB from food and humans. Finally, we discuss the effect of LAB on the gut microbiome on the basis of the currently available results from clinical trials and high-throughput sequencing data. In addition, we assess the availability and information retrievable from available genomic and metagenomic data for LAB from food and humans.

LAB diffusion and phylogenetic diversity

LAB are widely distributed in nutrient-rich habitats associated with food, plants, soil, animals and human hosts (Duar et al. 2017b; Wels et al. 2019). In recent years, the availability of a very large number of genomes of isolates from different sources has allowed comparative and evolutionary studies. Advancements made over the last few years were reviewed by (Duar et al. 2017b). In this work, the lifestyles of Lactobacillus sensu lato (i.e. including lactobacilli and related pediococci) were deduced by combining phylogenetic data with information about metabolism and from the literature. The > 200 species (Sun et al. 2015) were first grouped in main clades based on the phylogeny according to Zheng et al. (2015a) and then assigned to three main clusters: free living (i.e. associated with plant material or the environment without relying on a eukaryotic host), host adapted (i.e. specialized for living in association with eukaryotic hosts, with adaptive traits that facilitate persistence), and nomadic (i.e. with a dynamic, generalist lifestyle that involves both environmental and host niches, with no signs of specialization) (Martino et al. 2016). Interestingly, lifestyle allocation overlaps with phylogenetic grouping at both the species and subspecies levels, suggesting the occurrence of adaptive genomic evolution in different niches (Duar et al. 2017). To elaborate, the Lb. brevis, Lb. buchneri, Lb. collinoides, Lb. perolens, Lb. sakei, and Lb. vacinii groups were composed of species rarely found in animals and human hosts and therefore considered free living. Among the groups found to be nomadic, those species that, although not strictly autochthonous, exhibited adaptation to niches associated with humans or animals that could contribute to their persistence are of interest. These species could adapt to the gut and persist for at least a limited duration (Duar et al. 2017b). This is the case for Lb. casei/paracasei (Cai et al. 2007, 2009; Broadbent et al. 2012), Lb. plantarum (Siezen et al. 2010; Martino et al. 2016), and Lb. rhamnosus (Ribbera et al. 2013; Ceapa et al. 2015, 2016). Lb. amylovorus, Lb. iners, Lb. johnsonii, Lb. reuteri, Lb. ruminis, and Lb. salivarius were found to be adapted to vertebrate hosts, although some of them are also relevant in food fermentation (Vogel et al. 1999; Zheng et al. 2015b). The Lb. delbrueckii group comprised two main subclusters, one adapted to insects (e.g. Lb. bombicola, Lb. apis) and the other one to vertebrates (e.g. Lb. john-sonii, Lb. gasseri). Finally, the vertebrate gut was proposed as the real habitat of Lb. helveticus, despite its wide use in cheese production. Notably, host-adapted species or strains may have high ecological fitness in their respective hosts and therefore may be highly competitive when administered as probiotics (Duar et al. 2017a, b). On the other hand, species that did not undergo joint evolution with the host may be more appropriate for stimulating the immune system (Duar et al. 2017b). Notably, the lifestyle of pediococci remains unknown (Duar et al. 2017a), a problem that also exists for other LAB species that diverged from lactobacilli. The genus Streptococcus includes several pathogenic species, but the main food-related species, Streptococcus thermophilus, must have followed a divergent evolutionary path from that of its pathogenic relatives, and its genome has adapted to a well-defined and constant ecological niche, milk (Bolotin et al. 2004). This led to the loss of virulence factors and genes involved in the utilization of different carbohydrates, with the organism adapting to an environment in which the main carbohydrate source is lactose (Bolotin et al. 2004). Within the Lactococcus genus, Lc. lactis is of primary importance in the food industry. Lc. lactis taxonomy is currently based on phenotypic differentiation of two subspecies, Lc. lactis and Lc. cremoris. While the cremoris phenotype was found exclusively in dairy products and related environments, strains within the lactis subspecies have been isolated from different sources, including plants, vegetables and dairy environments (Wels et al. 2019). However, in Lc. lactis subsp. cremoris, a discrepancy between phenotype and genomic clustering was observed, and studies have shown that some strains with a cremoris genotype show a phenotype more similar to that of the lactis subspecies (Wels et al. 2019).

Other important LAB members are part of the family Leuconostocaceae, that includes heterofermentative microbes belonging to the genera Leuconostoc, Weissella, Oenococcus and Fructobacillus. Oenococcus and Fructobacillus were originally assigned to Leuconostoc genus, but were reclassified later, while Weissella includes several species previously classified as Lactobacillus or Leuconostoc spp. The genus Leuconostoc have been isolated from different environments, including plant material, roots, clinical sources and fermented foods, mainly vegetables and dairy, as well as chilled raw meat, where they may act as spoilage agents (Holland and Liu 2011).

Taxonomic classification has been traditionally based on phenotypic traits and sugar metabolism profiling, and subsequently coupled with 16S rRNA gene sequencing. However, the introduction of novel species, together with the widespread of genomic technologies, highlighted that the current LAB taxonomy should be revised. Indeed, several works based on genomic comparison showed that genetic similarity within Lactobacillus genus is as low as the value usually found for different orders or even classes (Sun et al. 2015; Parks et al. 2018; Salvetti et al. 2018). In addition, members of other genera (e.g. Pedococcus, Leuconos- toc, Weissella, Oenococcus) were shown to be intermixed among Lactobacillus species (Sun et al. 2015; Salvetti et al. 2018). Therefore, the Lactobacillus genus was proposed to be separated into 10 to 16 different genera (Pot et al. 2019). More recently, Zheng et al. (2020) showed that Lactobacillaceae and Leuconostocaceae families should be merged and suggested a reclassification of the genera included in these two families. Specifically, the emended Lactobacillus genus should incorporate only those species included in the Lb. delbrueckii group, while they proposed 25 novel genera enclosing other Lactobacillus species.

Although the urgent need for a reclassification was frequently highlighted and endorsed by an expert committee organised by the Lactic Acid Bacteria Industrial Platform (LABIP),
FERMENTED FOODS, PROBIOTIC LAB AND FUNCTIONAL FOODS

By transforming carbohydrates provided by the raw materials to mainly lactic acid, LAB have contributed to food quality and safety for decades, although this has occurred with highly variable degrees of human awareness. In fact, knowledge of the actual contribution and potential of LAB in food fermentation has evolved over time, from popular but uninformed use of fermentation to well-thought-out selection and application of LAB as starter cultures for the food industry.

FFs can be split into at least two major categories: (i) industrial and (ii) artisanal. In the first case, appropriately selected LAB cultures are employed as starter cultures to assure the technological outcome of the fermentation and in some other cases are used as specialized ‘adjuncts’ that are able to perform specific metabolic activities that support aroma production or texture development or add further value to the food product (Burns et al. 2012). The selected LAB cultures are meant to help achieve high reproducibility, quality and safety in highly controlled fermentation. Conversely, artisanal food fermentation is usually carried out with no starter or with naturally selected cultures. In the absence of starter addition, the LAB of environmental origin available in the raw materials can take guide fermentation and assist in product manufacturing and in obtaining the final FF. Although the composition of natural starter cultures is considerably influenced by the specific product and type of fermentation, these cultures are composed mainly of various species and strains of LAB that are specifically and naturally selected by the manufacturing process and whose composition is heavily influenced by raw materials and technological as well as environmental conditions. The spontaneously selected LAB in natural starter cultures are selected by a series of inoculation and refreshment steps in a traditional back-slopping procedure, where part of the fermented matrix of a previous manufacturing process is used as a natural starter in the fermentation process on the following day. The high diversity of FFs available across the globe is mirrored by the equally high microbial diversity of LAB employed daily in food fermentation.

The wide variety of raw material-microbe combinations results in thousands of different FFs and fermented beverages (Marco et al. 2017). Milk, meat, fish, vegetables, cereals and legumes can be fermented to obtain a variety of end products of high quality. Although the industrial use of selected LAB cultures has improved speed and quality standards, the number of FFs available and their associated microbial diversity has reduced. However, many countries across the world are currently promoting the use of FFs, especially traditional foods, for both hedonic (Xiang et al. 2019) and health-promoting properties (Chilton, Burton and Reid 2015).

Functional foods deliver additional or enhanced benefits over and above their basic nutritional benefits (Bell et al. 2018). LAB can contribute to rendering a FF functional, via both their presence and specific activities. While transforming raw materials through fermentation, LAB activity can indirectly confer several properties to FFs, making them valuable products for human health. In fact, beyond lactic and other acids, some metabolic activities and products can be developed during fermentation and confer interesting potential health-promoting properties to FFs (Şanlier, Gökçen and Sexgin 2019). Several observational studies have been performed to support this hypothesis and have linked the consumption of FFs (mostly yogurt) with beneficial effects on weight management (Mozaffarian et al. 2011), cardiovascular disease and type 2 diabetes (Chen et al. 2014; Tapsell 2015). Moreover, a link between FF consumption and mood and brain activity is also emerging (Tillisch et al. 2013; Aslam et al. 2018).

Several functional foods are recognized as such because they contain and deliver probiotic microorganisms. Many species and strains of LAB are regarded as probiotics, which are ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’, according to the definition proposed in 2001 by an expert panel working on behalf of the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) and subsequently endorsed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in the consensus statement of 2014 (Hill et al. 2014). The ISAPP confirmed that the term ‘probioic’ for food and food supplements should be used under certain conditions, including the administration of a minimum of 1 × 10⁸ CFU/day, a full genomic characterization of the probiotic strain and a history of safe use (Hill et al. 2014). Although a limited number of claims of health benefits of LAB have been approved, the probiotics market is thriving and is expected to grow further (Global Market Insight 2018). Probiotic strains are defined and potentially selected based on well-established criteria determined by the FAO and WHO (Araya et al. 2002). Strain identification, safety, stress tolerance and epithelial adherence capabilities are among the principal tests for screening probiotic strains (Pereira et al. 2018).

Owing to their food origin, some LAB species (mostly Lactobacillus and Streptococcus spp.) have a generally recognized as safe (GRAS) status according to the U.S. Food and Drug Administration (FDA) (https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices). In Europe, the concept of Qualified Presumption of Safety (QPS) was developed in 2007 by the EFSA to assist the safety assessment of microorganisms deliberately introduced into the food chain. The main difference between the GRAS and QPS concepts is that the former is generally limited to a specific application of a microorganism, while QPS refers to its generic safety in all possible uses. The QPS status evaluation is based on four points: taxonomy, scientific knowledge, the safety assessment (presence of virulence factors, production of toxins, antimicrobial resistance, reported cases of infection) and the expected end usage. When a species is included in QPS list, all the strains of that species will not need a full safety assessment (Sanders et al. 2010; Bourdichon, Lalund and Tenning 2019). Twenty-four Lactobacillus species, besides Lactococcus lactis, Streptococcus thermophilus and some Leuconostoc and Pediococcus species gained QPS status. Notably, no Weissella and Enterococcus spp. are included in this list (https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps). However, few cases of septicemia induced by lactobacilli are also reported, but this typically occurs only in patients with pre-existing health problems, such as immunocompromised (O’Callaghan and O’Toole 2013).

Among LAB, at least ten species of Lactobacillus and Lactococcus lactis have been shown to exhibit probiotic properties, and their importance as health-promoting bacteria together with novel non-LAB species and strains has been recently reviewed (Douillard and de Vos 2019).

Therefore, an additional benefit of FFs is that they are natural sources of LAB, and as such, they can be regarded as ‘naturally potential’ functional foods. Regardless of the origin of
the raw material, be it milk, vegetable or even meat, FFs can contain high loads of live LAB at the end of fermentation and in the final product. This does not apply simply to any FF. In fact, many foods obtained through fermentation do not contain live bacteria because they are inactivated by heat, as in the case of bakery products, or are physically removed, as in the case of alcoholic beverages (Rezac et al. 2018). Nevertheless, fermented milks, cheeses, fermented vegetables, meats, etc., do contain a considerable amount of live bacteria at consumption, which increases the number of microbes in the diet by up to 10,000-fold (Lang, Eisen and Zivkovic 2014). Diets rich in FFs offer remarkable microbial exposure in contrast with highly processed foods provided in societies with a high level of Westernization and hygienic practices. Rezac et al. (2018) surveyed the amount of live LAB occurring in a variety of FFs at retail and found loads ranging between $10^5$ and $10^9$ CFU/g or ml, with dairy products containing the highest levels. Such high amounts of live LAB are therefore ingested with FFs and reach the human gastrointestinal tract (GIT). What happens after ingestion depends on the specific genetic and functional traits of the LAB strains and on their ability to resist the stress conditions to which they are exposed. High concentrations of pepsin and low pH (<3) are the principal barriers in the stomach, while bile and pancreatin are the typical adversities encountered in the small intestine. However, if they are able to endure to such stress factors, these bacteria can reach the colon and join the complex environment of the gut microbiome (see below).

Fermented foods as source of microbial metabolites

Overall, the numerous enzymatic activities that can be carried out during food fermentation by LAB can change the biochemical composition of foods, releasing bioactive compounds that can provide health-promoting properties that the same matrix would not display without fermentation (Marco et al. 2017). Indeed, some LAB strains may exert health-promoting activity even if inactivated. The term ‘postbiotic’ was recently coined, indicating microbial metabolites or components of bacterial cell walls released in a matrix from which microbes are removed or inactivated and conferring health benefits when administered in sufficient amounts (Aguilar-Toalá et al. 2018). Such compounds include β-galactosidase for improved lactose digestion; conjugated linoleic acid, bioactive peptides and polyamines; and phenolic compound derivatives for oxidative stress improvement (Marco et al. 2017). LAB can also produce exopolysaccharides (EPSs) with potential cholesterol-lowering, antioxidative, antioxidant, and immunomodulatory properties (Nampoothiri et al. 2017; Şanlıer, Gökcen and Sezgin 2019). Several LAB produce B-group vitamins during fermentation and can effectively increase vitamin levels (LeBlanc et al. 2011). For example, Lb. casei KNE-1 was shown to synthesize thiamine ($B_1$) and riboflavin ($B_2$) in fermented milk drinks, while some strains of S. thermophilus, Lb. delbrueckii and Lb. amylovorus can be used to produce yogurts or fermented milks that are naturally rich in folate ($B_9$), which is particularly important during pregnancy (Linares et al. 2017). In addition, some Lc. lactis strains can produce menaquinone ($K_2$) in cheese and kefir (Walther et al. 2013). Other strains can produce neuroactive molecules, among which gamma-aminobutyric acid (GABA) is the most well studied. GABA acts as a neurotransmitter in mammals and performs additional functions, such as lowering blood pressure, relaxing muscles, and reducing psychological stress (Pessione and Cirrincione 2016; Şanlıer, Gökcen and Sezgin 2019). The ability to produce GABA is recognized in several bacteria of gut origin (Wall et al. 2014), but fermented products rich in GABA have also been developed using specific strains of Lb. casei, Lb. plantarum, S. thermophilus, Lb. brevis and Lc. lactis as starters in fermented dairy products, legumes, cereals, and chocolate (Pessione and Cirrincione 2016; Linares et al. 2017).

Moreover, LAB can release biologically active peptides via proteolysis (Linares et al. 2017). The most well-studied peptides are antihypertensive peptides that can regulate blood pressure through inhibition of angiotensin-I-converting enzyme (ACE) and have been proposed as natural alternatives to antihypertensive drugs. ACE-inhibiting peptides are found mainly in fermented dairy products and fermented vegetables or legumes and are produced by several LAB used as starter cultures in FFs, including strains of Lb. helveticus, Lb. casei, Lb. delbrueckii, Lb. plantarum, Lc. lactis, and S. thermophilus (Shakerian et al. 2015; Li et al. 2017). In addition, peptides with different activities, such as anti-inflammatory, antioxidant, immunomodulatory, and antimicrobial activities, have also been identified in FFs (Pessione and Cirrincione 2016).

Another class of health-promoting molecules produced in FFs is conjugated fatty acids derived from bioconversion of linoleic acid (conjugated linoleic acid, CLA). CLA is naturally present in ruminant milk due to the activity of rumen bacteria, but the amount is by far sufficient to show some effects (Linares et al. 2017). Indeed, several LAB are known to produce CLA in milk products (Lc. lactis, Lb. acidophilus, Lb. casei, Lb. plantarum, Lb. rhamnosus, Lb. delbrueckii), and their use as starter or adjunct cultures may be a promising strategy for the production of enriched biofunctional foods (Linares et al. 2017).

Finally, LAB may reduce the presence of anti-nutritional compounds in FFs. An example is the phytase activity of some LAB. Phytic acid is present in several foods of vegetable origin, including cereals and legumes, and is considered an anti-nutrient substance since it can form complexes that chelate various minerals, thus reducing their bioavailability (Sharma et al. 2020). Phytase-producing LAB are able to hydrolyse phytates and release minerals. Different strains of Lb. plantarum, Lb. amylovorus, and Lb. acidophilus have been used for fermentation of sourdough from wheat, rye and oat; soy-based products; and beer and were able to reduce phytate concentrations in the fermented matrix (Sharma et al. 2020).

Besides exerting health-promoting activities and producing beneficial metabolites, some LAB strains are recognized as the main producers of biogenic amines (BA) in fermented foods from amino acids decarboxylation (Barbieri et al. 2019). The consumption of products containing high levels of BAs, depending on individual sensitivity or the concomitant assumption of specific drugs or ethanol, can cause headache, heart palpitations, vomiting, diarrhea and hypertensive crises (Barbieri et al. 2019). Moreover, several LAB strains have been shown to carry out genes responsible for antibiotic or antimicrobial resistance, that might be transferred to pathogens or GIT microbes (Campedelli et al. 2019). Therefore, in-depth and rigorous genomic characterization of food-related LAB strains is desirable to identify the presence of potentially dangerous activities.

LAB prevalence and diversity in fermented foods

Fermentation has been traditionally used as an empirical method to improve food stability, and in recent years, it has been used to enhance the flavour, texture, and functional properties of food (Dimidi et al. 2019). LAB from several genera are commonly predominant in FFs, but other bacteria (e.g. propionibacteria and acetic acid bacteria), as well as fungi, also contribute to specific food fermentation processes.
S. thermophilus, Lc. lactis, Leuconostoc species, and several Lactobacillus species are the LAB most commonly found in FFs, either as naturally occurring bacteria or deliberately added as starter cultures. These species are among the most common commercially used bacteria, contributing to the production of yogurt, kefir, cheese and other dairy products; sauerkraut, kimchi and pickles; cured meat and fish; sourdough-based baked products; and many other traditional fermented foodstuffs around the world (Tamang et al. 2020). The main metabolic activity of interest for food production is the ability of these bacteria to carry out lactic acid fermentation, an anaerobic process that converts pyruvate molecules from glycolysis to lactic acid (homolactic fermentation) or lactic acid and other compounds, such as acetic acid, ethanol, and CO$_2$ (heterolactic fermentation). These species can also activate several secondary metabolic processes that lead to the production of flavour compounds or typical textures. Combination of these metabolic processes leads to hundreds of different products, some of which are globally widespread, while many others are locally produced, often according to a traditional manufacturing practice (Chilton, Burton and Reid 2015; Tamang et al. 2020). Different food matrices can be considered specific ecological niches in which well-adapted LAB species finalize the fermentation process. In recent years, hundreds of studies have described microbial dynamics during the fermentation of different foodstuffs by high-throughput sequencing (HTS), extensively reviewed elsewhere (Ercolini 2013; De Filippis, Parente and Ercolini 2017, 2018b). Most of these studies are based on amplicon sequencing of taxonomically relevant genes and merely provide a survey of the microbial diversity occurring during food fermentation. Most of these studies have been collected in the FoodMicrobiomnet repository (http://www.foodmicrobiomnet.org; Parente et al. 2016; Parente et al. 2019). FoodMicrobiomnet contains data on microbial taxonomic composition from 44 HTS studies on food microbial ecology, including 29 datasets on food fermentation. To date, this repository includes a total of 2234 samples from food or food environments covering dairy, meat, fruits, vegetables, cereal-based foods, and ready-to-eat foods, with 806 samples of FF products. The samples are labelled according to the FoodEx classification (http://www.efsa.europa.eu/en/data/data-standardisation). Due to the availability of an app built with the Shiny R package, even inexperienced users can easily explore the data, access external resources, filter samples based on multiple predefined criteria, aggregate samples and bacterial taxa, extract the taxonomic composition of specific groups of samples, and use them in comparative studies. We considered 806 samples spanning multiple FF matrices, extracted the prevalence of different LAB genera and species (collated in taxonomic groups, as defined by Salvetti et al. 2018), and grouped them according to the type of product or ripening time (Fig. 1). The niche specificity of Lactobacillus species is highlighted: Lb. delbrueckii group is prevalent mainly in dairy products, while the Lb. plantarum and Lb. sakei groups showed 100% prevalence in fermented vegetables and meat samples, respectively. Lb. buchneri group (including Lb. buchneri, Lb. sanfranciscensis, Lb. brevis) prevailed in sourdough, where Lb. sanfranciscensis is a well-known member of the microbial community (Ripari, Gänzle and Berardi 2016). Among other LAB genera, Weissella is found exclusively in naturally leavened sourdough, while Streptococcus and Lactococcus are found in cheeses and kefir. In addition, while most fresh and short-ripened cheeses contain thermophilic LAB, such as Streptococcus, high variability in LAB composition was found in ripened cheeses, in which mesophilic lactobacilli and Lactococcus are also present (Fig. 1). Some commonly consumed dairy products with a simple and defined microbiota structure (i.e. yogurt) are obviously not considered in Fig. 1 as they have not been studied by HTS approaches. LAB are often deliberately used for inoculation to start fermentation, as either selected commercial cultures or natural starters obtained according to a back-slopping procedure. Nevertheless, several artisanal products are fermented without the addition of starter microbes, but they arise from raw materials or from the facility environment, equipment and tool surfaces. Indeed, the food processing environment harbours a resident and complex microflora that can be transferred to the product and represent a primary source of beneficial LAB (Montel et al. 2014; Stellato et al. 2015; Bokulich et al. 2016). Unfortunately, taxonomic identification at the species level is often not achievable with common amplicon-based HTS technologies, and many studies have reported genus-level identification (Fig. 1). This is a substantial limitation considering the wide species and subspecies diversity existing within LAB and the specific roles that these microbes can play during food production. This limitation may be overcome using a complex shotgun HTS approach, which remains underexploited in food-related microbiome studies. The use of metagenomics can be of invaluable importance for the identification of microbial genes and pathways leading to the production of metabolites associated with the typical sensorial profile of specific FFs, as well as for detecting potential health-related activities (De Filippis, Parente and Ercolini 2018b). In fact, in addition to producing lactic acid during fermentation, LAB confer important desirable properties to FFs. By degradation of carbohydrates, proteins and lipids, LAB can synthesize molecules positively associated with the flavours of FFs or modify the texture of some products by proteolysis, lipolysis or EPS production (Galle and Arendt 2014; Di Monaco et al. 2015; De Filippis et al. 2016; Gänzle and Ripari 2016; McAuliffe, Kilcawley and Stefanovic 2019). Nevertheless, it should be pointed out that identification by HTS does not imply that the microbes are alive at the moment of consumption, since these studies are usually based on DNA, which may be derived from dead or inactive cells. Viable counts of LAB in several FFs have also been reported (Rezac et al. 2018). It is estimated that between 10$^6$ and 10$^{12}$ CFU of bacteria may be ingested daily with the consumption of FFs (Derrien and van Hylckama Vlieg 2015). The quantities of S. thermophilus and Lb. delbrueckii in commercial yogurts and fermented milk vary from 10$^4$ to 10$^9$ CFU/ml, while the abundance of lactobacilli in cheeses ranges from 10$^3$ to 10$^9$ CFU/g, decreasing during ripening (Rezac et al. 2018). The levels of LAB in fermented sausages were reported to vary according to the origin, with fermented sausages from Europe showing higher counts than those from the US (<10$^6$ vs 10$^9$ CFU/g), which is probably associated with the more artisanal manufacturing process used for European products (Rezac et al. 2018). Therefore, the real amount of LAB ingested through a specific FF may also be extremely variable according to the geographical origin, manufacturing process (e.g. artisanal vs industrial; presence, length, and conditions of ripening; etc.), time and type of storage, and use of inactivation steps before consumption. For example, bread and other baked goods are usually cooked, while several fermented vegetables are pasteurized before commercialization to improve stability. However, the low levels of live microbes in the final product do not preclude a positive functional role. Indeed, several LAB may produce vitamins or other bioactive molecules in situ or inactivate anti-nutritional factors, thus exerting a positive health effect even if not alive at the time of consumption (Linares et al. 2017; see above).
THE GUT MICROBIOME

The gut microbiome is among the most complex known microbial communities and among the most well studied. It comprises a very large variety of microbial strains belonging to species of bacteria, archaea, fungi and viruses that live in close relationship with the human host and whose combined genome harbours at least 100 times as many genes as the human genome (Bäckhed et al. 2005; Belkaid and Hand 2014). Members of the gut microbiome can influence host health through the production of a wide variety of beneficial or detrimental metabolites, and such molecules can be derived from both metabolic intermediates of the host and dietary precursors (Holmes et al. 2012; De Filippis et al. 2018a; Roager and Dragsted 2019). The most recent research advances have shown a high potential impact of the gut microbiome on the regulation of the equilibrium between health and disease, which is due to both the composition and functions of the microbiome. Complexity in composition is one of the main features of the gut microbiome, and microbial richness in terms of both species and genes has been linked to health (Cotillard et al. 2013; Le Chatelier et al. 2013; Vangay et al. 2018). In addition to differences in microbial composition based on health, lifestyle and geography (Almeida et al. 2019; Nayfach et al. 2019; Pasolli et al. 2019), the gut microbiome is characterized by high inter-individual variability (Truong et al. 2017). However, a large proportion of bacterial species are present in each individual and likely constitute a resilient microbial community (Aguirre de Cárder 2018). In light of these considerations, the possible role of probiotics appears even more challenging, as after overcoming the barriers presented by the stomach and small intestine, probiotics encounter an army of hundreds of different species and strains to compete with, which affects their chances of exerting their beneficial effects. While the host-specific microbial community can be considered a resident microbiome, the microbes that we ingest and that reach the colon can be regarded as a transient microbiome, the composition of which depends on the type of exposure and on the type of food in the case of foodborne microorganisms (Derrien and van Hylckama Vlieg 2015). Indeed, the gut microbiome is exposed daily to microbes from the external environment, which are mainly of food origin. Probiotic LAB strains can be part of such a transient community and are supposed to perform their activity during passage through the gut and in the presence of the other members of the gut community.

Currently, it remains unknown which fraction of the food microbiome is actively transferred to the intestine and what
type of activity those strains can exert in such a complex ecosystem. In fact, there is little literature on the prevalence of LAB in FF consumers and non-consumers. In addition, the possibility that non-probiotic food-borne LAB can also be transferred to the gut to a certain extent is currently underexplored, as are the actual activities that LAB can carry out in the gut as a complementary transient microbial community.

LAB in the human gut

LAB are also widespread in other nutrient-rich environments, among which the human host is of great interest due to the potential functional properties of these bacteria (Soomro, Masud and Anwaar 2002; Durar et al. 2017b). The human microbiome interacts continuously with microbes originating from external environments, including food-origin sources. Probiotic LAB species and strains can constitute a portion of this transient microbiome and perform their activities during their transition in the gut, in addition to non-probiotic LAB that can potentially be transferred into the gut to a certain extent. Despite the availability of abundant nutrients, LAB present in the gut have to deal with a challenging scenario that involves hundreds of different bacterial and non-bacterial species sharing the same habitat (Pessione 2012).

Despite the long-term efforts in characterizing LAB, characterization of the effective contribution of these bacteria to the human microbiome remains a major challenge, and contradictory results have been reported in the literature (Walter 2008; Pessione 2012; George et al. 2018). Going back a hundred years, seminal studies identified lactobacilli, which we focus on here due to their prevalence in the LAB literature, among the most prevalent and abundant microorganisms in the human gut (Tannock 1999). At that time, techniques to cultivate anaerobic organisms were not yet available, which likely led to overestimation of the more easily cultivable microbes such as lactobacilli, while most of the gut (anaerobic) microbes remained undetected, a problem that despite continuous technological advancements has not yet been fully resolved (Almeida et al. 2019; Nayfach et al. 2019; Pasolli et al. 2019). For a long time, lactobacilli were considered to be numerically relevant members of the microbiome (Walter 2008), but most of the research conducted in the last few decades found that these bacteria are subdominant and therefore represent instead a small fraction of the overall microbiome composition. When using total anaerobic culturing techniques, the amount of lactobacilli rarely exceeded 10^8 CFU/g and accounted for an average of 10^6 CFU/g of intestinal content (Mitsuoka 1992; Walter et al. 2001; Dal Bello et al. 2003; Walter 2008), which represented a small fraction (~0.01%) of the total count assuming that the intestinal content can reach up to 10^12 CFU/g (O’Hara and Shanahan 2006). However, cultivation-based approaches may also be affected by biases because while lactobacilli can be easily isolated from food, isolation from human stool samples is more difficult since bifidobacteria are much more abundant and share similar nutritional requirements (Quartermiri et al. 2016). On the one hand, findings obtained from culture-based approaches were confirmed in multiple studies by culture-independent methods (Walter 2008), which included, for example, fluorescent in situ hybridization (FISH) in combination with fluorescence microscopy (Harmsen et al. 2002), quantitative real-time PCR (Rinttilä et al. 2004), and high-throughput analysis of 16S rRNA gene amplicon sequencing data (Suau et al. 1999; Hayashi, Sakamoto and Benno 2002; Hold et al. 2002; Eckburg et al. 2005). The FISH data showed a relative abundance for the Lactobacillus/Enterococcus group ranging from 0.01–1.8% (Flint 2006; Louis et al. 2007). However, the results of other studies differed from these ones, instead finding that LAB occurrence may not be negligible in the human gut. This was mainly driven by advancements in 16S rRNA and metagenomics methodologies (Quince et al. 2017b), which made it possible to obtain largely unbiased perspectives on the relative importance of LAB in the context of the other members of the gut microbiota (Heeney, Gareau and Marco 2018). Approximately 5% and 13% of the sequences from 16S rRNA libraries were attributed to lactobacilli by (Frank et al. 2007) and (Hayashi et al. 2005), respectively. Using 16S rRNA data, Lactobacillus were estimated to constitute on average 6% of the bacterial cells in the duodenum (Nistal et al. 2016) and 0.3% in the colon (Almonacid et al. 2017). A longitudinal metagenomic study surveyed lactobacilli in a single person at three timepoints and found 52 sub-dominant species, 80% of which were detected in a two-year timeframe (Rossi et al. 2016). These results suggested that a relevant LAB population may be harboured in the human gut, with consistent inter-individual variations that may be driven by multiple factors, with the most likely one represented by diet (David et al. 2014) and the ingestion of LAB-enriched foods. Notably, most of this literature was derived from studies on faecal material, while very little is known about the small intestine microbiome due to it being accessible via only invasive procedures (Derrien and van Hylckama Vlieg 2015; El Aidy, van den Bogert and Kleerebezem 2015; Stolaki et al. 2019). This may represent an overlooked scenario because consumption of a dose of 10^10 bacterial cells may have a strong influence, at least temporally, on the microbial composition of the small intestine, since the microbial density in this organ, ranging from 10^4 to 10^6 bacteria/ml, is much lower than that in the colon (Derrien and van Hylckama Vlieg 2015).

Along with quantification of the LAB community in the gut, of comparable or even greater importance is the discrimination between resident (defined as autochthonous) and transient (allochthonous) components. This task is not trivial since LAB are continuously administered in the human ecosystem through ingested food and therefore represent a rather peculiar microbial group (Walter 2008; Rossi et al. 2016). Notably, populations of allochthonous species may appear stable if introduced regularly into the habitat (Duar et al. 2017b). Seminal studies on this topic were well summarized by (Walter 2008). Pioneering studies (Lerche 1961; Reuter 1965; Mitsuoka 1969) found transient and resident lactobacilli strains in stool samples, with the latter ones identified as Lb. crispatus, Lb. gasseri, Lb. reuteri, Lb. ruminis, and Lb. salivarius (Mitsuoka 1992; Reuter 2001). Further studies showed that a large fraction of the LAB species found in the gut are probably allochthonous and do not form stable populations, along with other species that can be considered autochthonous members of the microbiome (Tannock, Munro and Harmsen 2000; Walter et al. 2001; Walter 2008). For example, Lb. ruminis and Lb. salivarius were found to be persistent in multiple subjects for more than 18 months (Tannock, Munro and Harmsen 2000). A list of 17 lactobacillus species typically found in the gut was reported by (Walter 2008), comprising Lb. acidophilus, Lb. brevis, Lb. casei, Lb. crispatus, Lb. curvatus, Lb. delbrueckii, Lb. fermentum, Lb. gasseri, Lb. johnsonii, Lb. paracasei, Lb. plantarum, Lb. reuteri, Lb. rhamnosus, Lb. ruminis, Lb. sakei, Lb. salivarius, and Lb. vaginalis, most of which were identified as allochthonous members. This list was similarly reported by (Vaughan et al. 2002 and O’Callaghan and O’Toole 2013) and integrated with other LAB species. Some studies have also verified the colonization abilities of specific LAB strains. As an example,
two strains of L. mucosae and L. reuteri reached higher population levels and were recovered more frequently from faecal samples than a strain of L. acidophilus (Frese, Hutkins and Walter 2012). Other studies determined the extent to which a host’s persistent gut microbiota influences niche permissivity to transient LAB (Zhang et al. 2016) and how invasion by transient LAB can perturb the stability of microbial ecosystems (Amor, Ratzke and Core 2019).

The quantities of LAB species that are persistent in the gut may be larger than currently documented. In the aforementioned work (Rossi et al. 2016), more than 40 species were detected in a single person in a two-year timeframe, indicating the need to conduct more and much larger analyses in similar settings.

Some untargeted studies have shown variations in the proportions of LAB in the gut and found positive or negative correlations with disease or chronic conditions. Depletion of intestinal lactobacilli was frequently associated with disease. As summarized in (Heeney, Gareau and Marco 2018), Lactobacillus was depleted under conditions of type 1 diabetes (de Goffau et al. 2014; Alkanani et al. 2015), irritable bowel syndrome (Liu et al. 2017; Zhuang et al. 2017), multiple sclerosis (Chen et al. 2016), human immunodeficiency virus infection (Yang et al. 2016), and prenatal stress (Zijlmans et al. 2015). On the other hand, enrichment of lactobacilli was verified in conditions of Crohn’s disease (Wang et al. 2014; Lewis et al. 2017) and rheumatoid arthritis (Zhang et al. 2015). Contradictory findings were reported for type 2 diabetes (Karlsson et al. 2013; Forslund et al. 2015) and obesity (F. S. Teixeira et al. 2013; Ignacio et al. 2016).

Notably, most of the previous research has been devoted to the characterization of lactobacilli, while less attention has been given to other LAB members (Van den Bogert et al. 2013; Mignon et al. 2016). Additionally, there is a lack of research aimed at assessing the distribution of LAB in the global population. This gap may be bridged by taking advantage of the growing availability of HTS data, as we will show below.

**DATABASES AND COMPUTATIONAL TOOLS TO RETRIEVE GENOME-WIDE INFORMATION ON THE PREVALENCE AND FUNCTIONAL DIVERSITY OF LAB FROM DIFFERENT SOURCES**

Food and microbiome research can take advantage of the continuous improvement in HTS technology, which has revolutionized the microbial ecology field in the last two decades (Goodwin, McPherson and McComb 2016). The continuous decrease in sequencing cost has been associated with exponential growth in terms of the number, diversity, and complexity of the sequenced data. Large international consortia have been established to mainly characterize the human microbiome (Human Microbiome Project (Human Microbiome Project Consortium 2012) and MetaHIT (Qin et al. 2010), along with other initiatives such as the Tara Oceans Program (Sunagawa et al. 2015), the MetaSUB Consortium (MetaSUB International Consortium 2016), and the Earth Microbiome Project (Thompson et al. 2017), while such large efforts are still lacking in the food microbiome field.

Currently, two main approaches can be adopted in the microbiome field. The 16S rRNA gene sequencing method profiles selected organisms or single marker genes (Hamady and Knight 2009). It is the most cost-effective method, and the main output is limited to the generation of taxonomic profiles, typically at the genus level. Complete pipelines have been developed and widely used (Schloss et al. 2009; Bolyen et al. 2019), in addition to additional newly proposed methods (Callahan et al. 2016). More advanced analyses include oligotyping to obtain species- or even strain-level resolution (Eren et al. 2013) and (rough) estimation of functional potentials (Langille et al. 2013). Different repositories with annotated reference sequences have been made available and continuously updated (Pruesse et al. 2007; McDonald et al. 2012; Cole et al. 2014; Yoon et al. 2017). In addition, curated databases dedicated to specific environments of interest have been developed, for both 16S rRNA gene sequences and whole microbial genomes. DAIRYdb (Meola et al. 2019) provides a manually curated repository of 10,290 full-length 16S rRNA gene sequences from prokaryotes tailored for dairy product analyses. In addition, Almeida et al. (2014) developed a curated genome catalogue of 137 microbial species isolated from dairy products. Higher resolution can be obtained by acquiring the entire genomic content of a sample through (shotgun) metagenomic sequencing (Quince et al. 2017b). The large compendium of tools developed for this technique can be grouped into two main approaches, i.e. mapping-based profiling and de novo assembly. Species-level taxonomic profiles can be generated by adopting different mapping-based methods (Sunagawa et al. 2013; Wood and Salzberg 2014; Truong et al. 2015). Additional tools have also been developed to reduce errors by taking advantage of environmental and domain-specific information, which is however a quite overlooked research topic. This is the case of the methodology developed in (Seol et al. 2019) and aimed at reducing errors in terms of false positive rate for the specific identification of LAB and probiotic species.

Recently, attention has also been given to methodologies for metagenomic analysis with strain-level resolution. Different techniques have been proposed and are mainly based on the detection of single-nucleotide variants (SNVs) in the core genes (Costea et al. 2017; Truong et al. 2017), the identification of unique combinations of genes in the pangeneome of a species (Scholz et al. 2016), or the use of a combination of these methods (Nayfach et al. 2016). Attempts have also been devoted to resolving multiple strains of the same species in a single sample (Quince et al. 2017a), although this remains an unresolved challenge along with the profiling of low-abundance non-dominant strains (Segata 2018). In addition to providing information on taxonomic composition, metagenomics can also be used for functional profiling (Franzosa et al. 2018). Complementary to mapping-based approaches is de novo metagenomic assembly. This method aims to provide (draft) genomes (defined as metagenome-assembled genomes, MAGs) of the microbial members present in samples. It can be used to expand the set of genomes of known and already studied species, but at the same time, due to its reference-free nature, provides the possibility to identify and characterize unknown members of the microbiome. Notably, MAGs can be integrated with genomes reconstructed from isolates and post-processed with a myriad of procedures based on comparative genomics that are quite standard for genomes from isolates. The idea of obtaining genomes directly from metagenomes is not new (Allen and Banfield 2005); however, this method was rarely applied until a few years ago due to computational challenges that have been addressed only recently. First, raw reads are assembled into contigs, with metaSPades (Nurk et al. 2017) and MEGAHIT (Li et al. 2015) representing the two most widely used tools. Then, the contigs are grouped into (draft) genomes through binning, with the popular tools represented by CONCOCT (Alineberg et al. 2014), MetaBAT2 (Kang et al. 2019), and DAS Tool (Sieber et al. 2018). Finally, only genomes of sufficient quality (usually evaluated in terms of completeness...
and contamination through tools such as CheckM (Parks et al. 2015) and BUSCO (Simão et al. 2015)) are retained and constitute the final set of genomes. Different papers devoted to the reconstruction and characterization of MAGs from large-scale scenarios have been recently published. Of great relevance is the characterization of the human microbiome (Almeida et al. 2019; Nayfach et al. 2019; Pasolli et al. 2019) along with the microbiomes, for examples, from the rumen (Stewart et al. 2018, 2019), non-human primates (Manara et al. 2019), and multiple other environments (Parks et al. 2017). However, similar efforts in the food microbiome field are still lacking.

The growing number of publicly available microbiome datasets enables hypothesis testing for environmental niches as well as meta-analyses across multiple studies. However, different factors prevent the research community from taking full advantage of these resources. These barriers include the need for substantial investment of time, computational resources and specialized bioinformatic expertise as well as inconsistencies in annotation and formatting between individual studies. To overcome these issues, in the last few years, several efforts have been devoted to the creation of resources and databases for the release of different types of microbiome data, which represent invaluable resources that allow the community to integrate newly acquired data with existing data. Comprehensive resources for both 16S rRNA and metagenomic data are represented by MGnify (Mitchell et al. 2020) and QIITA (Gonzalez et al. 2018). These resources integrate both the deposition of sequence data and distribution of products derived from multiple post-processing pipelines. Currently, MGnify (formerly EBI Metagenomics) integrates 214,977 samples spanning 3685 studies and is associated with six main biomes, i.e. the aquatic, food production, human, plant, soil, and wastewater biomes. While more than 40% of the samples are associated with the human microbiome, only a tiny fraction (1%) is related to food production systems. QIITA includes an even larger number of samples (i.e. 232,651 public and 137,644 private samples), although this resource is much more focused on 16S rRNA data. Additionally, in this case, very few public studies are associated with food.

Other resources of smaller size have also recently been made available and are focused on the collection, curation, and processing of samples derived from specific biomes and data types. A representative example of a resource focused on 16S rRNA data is FoodMicrobionet (Parente et al. 2019), already introduced above, which aims to retrieve and combine information specifically from food bacterial communities. Another interesting platform, although not specifically food focused, is Integrated Microbial Next Generation Sequencing (IMNGS (Lagkouvardos et al. 2016)). All prokaryotic 16S rRNA datasets available in Sequence Read Archive (SRA), which is the major database with permanent storage and public access to DNA sequencing data, which is the major database with for both 16S rRNA variable regions, integrated with phylogeny, taxonomy, and public participant data of the Human Microbiome Project (HMP). This is a good example in which, by removing the hurdles of data access and management, researchers with only basic R skills can analyse HMP data in a quick and simple way (Human Microbiome Project Consortium 2012).

Similar efforts to build databases for the human microbiome have also been conducted for shotgun metagenomics data. One example is the curatedMetagenomicData package (Pasolli et al. 2017), which currently includes more than 10,000 metagenomes from approximately 50 studies. This tool provides uniformly processed microbiome data, including bacterial, fungal, archaeal, and viral taxonomic abundances, in addition to quantitative metabolic functional profiles and standardized per-participant metadata. As in the case for HMP16SData, the data resources are accessible to users with minimal bioinformatic knowledge, and integration with the R/Bioconductor environment allows flexibility for researchers to perform novel analyses and methodological development and for integration of resources. Other similar resources that have been developed subsequently are Microbiome Learning Repo (ML Repo (Vangay, Hillmann and Knights 2019)) and Data Repository for Gut Microbiota (GMRepo (Wu et al. 2020)). ML Repo is a public, web-based repository of 33 curated classification and regression tasks from 15 already published datasets. GMRepo contains 58,903 human gut samples (17,618 from metagenomics and 41,285 from 16S rRNA data) spanning 253 datasets associated with 92 main phenotypes. In this case, the collected samples are organized according to their associated phenotypes. This tool is equipped with a graphical query builder, enabling users to make customized, complex and biologically relevant queries to obtain relevant information that is easy to access. Although such resources are related to the human microbiome in wide terms, at the same time they can be of interest for researchers interested in characterizing LAB in the human gut. Example of database developed for different biomes is the TerrestrialMetagenomeDB for terrestrial metagenomes (Corrêa et al. 2020), while similar products have not yet been developed for metagenomic studies from food microbiomes.

**AVAILABILITY OF LAB GENOMES IN FOOD AND THE GUT FOR COMPARATIVE STUDIES**

Along with the availability of databases to improve the accessibility to raw sequences and their integration with metadata information, access to genome assemblies is also of great relevance. The benchmark in this context is the NCBI Assembly database (Kitts et al. 2016), which provides stable accessioning and data tracking for genome assembly data. Data can be found for different structures, such as sets of unordered contigs or scaffold sequences, bacterial genomes, or more complex structures, such as human genomes. A particular version of an assembly is identified unambiguously, and track changes are kept to identify genome updates. Along with the nucleotide sequences, this resource provides metadata such as assembly names, statistical reports of the assembly, and assembly update history. Users can easily download sequences and annotations through the NCBI Genomes FTP site.

By searching the NCBI Assembly database with the keyword ‘prokaryotes’, we found 223,803 genomes. Filtering by taxonomy, we identified 3525 (1.6%) genomes associated with LAB species, namely, Lactobacillus (N = 2748), Lactococcus (N = 288), Leuconostoc (N = 204), Pedicoccus (N = 96), S. thermophilus (N = 62), and Weissella (N = 129) (Fig. 2A and Supplementary Table S1). These
genomes were associated with 257 taxa (Fig. 2B and Supplementary Table S2), with Lb. plantarum (N = 473), Lc. lactis (N = 223), Lb. rhamnosus (N = 191), Lb. paracasei (N = 183), and Lb. reuteri (N = 178), representing the most frequently occurring species. The first deposited strain was Lc. lactis subsp. lactis IL1403 in 2001 (Bolotin et al. 2001). The exponential growth of the number of available genomes is represented by the 80% increase in the number of deposited LAB genomes in the last 5 years (Fig. 2). This large number of public genomes represents a fundamental resource for mapping-based computational tools and for comparative genomics. For example, these genomes were used to propose a genome-based reclassification of the genus Lactobacillus (Wittouck, Wuyts and Lebeer 2019; Zheng et al. 2020) due to inconsistencies in the current taxonomy (Wuyts et al. 2017). The same approach was also recently applied to publicly available bacterial and archaeal genomes (Parks et al. 2019).

As reported above, LAB are widespread in natural environments. Considering the genomes from NCBI for 18 main LAB species frequently found in FFs or probiotic supplements, we summarized their source of isolation in Fig. 3 as reported in NCBI or in the linked publications. FFs are the primary source of isolation for several LAB strains. In addition, host-adapted species can be identified. Lb. gasseri, a well-known probiotic species, was mainly isolated from the human infant gut, while Lb. johnsonii, Lb. reuteri, and Lb. salivarius were also retrieved from other animal hosts, both mammals and birds (Fig. 3). Nomadic lactobacilli (i.e. Lb. casei, Lb. paracasei, Lb. plantarum, and Lb. rhamnosus) were isolated from a variety of sources, including human, animal, and insect hosts, as well as soil and plant material (Fig. 3). Indeed, genomic comparisons highlighted that these species usually have a large genome size and a large number of coding sequences, allowing them to adapt and survive in a wide range of environments (Duar et al. 2017).

Along with the growing availability of reference genomes from isolated sequences, the number of MAGs retrieved from metagenomic datasets is continuously increasing, and these data can be combined for comparative genomics. To explore the availability of reconstructed genomes from LAB, we considered the large set of MAGs (N = 154,723) retrieved from 9428 human metagenomes that was clustered in 4390 species-level genome bins (SGBs) based on a 5% genetic diversity (Pasolli et al. 2019, 2020). Forty-nine of these SGBs belonged to LAB species, for a total of 830 reconstructed MAGs, grouped as follows: Lactobacillus (37 SGBs, 515 MAGs), Lactococcus (4 SGBs, 49 MAGs), Leuconostoc (3 SGBs, 7 MAGs), Pediococcus (2 SGBs, 5 MAGs), S. thermophilus (243 MAGs), and Weissella (2 SGBs, 11 MAGs) (Supplementary Table S3). These numbers are correlated with the occurrence of such species in the human gut, although the prevalence of low-abundance microbes is underestimated due to the technical impossibility of reconstructing MAGs from metagenomes in such cases. A large majority of the SGBs (44, 89.8%; for a total of 823 MAGs) represent at least partially known SGBs (kSGBs) that include one or more isolate genomes available in public databases. The most extensively reconstructed kSGBs were those of S. thermophilus (243 MAGs), Lb. ruminis (145 MAGs), Lb. mucosae (50 MAGs), Lb. salivarius (42 MAGs), and Lb. rhamnosus (32 MAGs). Only 7 MAGs spanning 5 SGBs were associated with unknown species (kSGBs), defined as SGBs lacking any publicly available genomes from isolate sequencing, which suggests the rarity of as-yet-uncharacterized LAB species in the human microbiome. Notably, reference genomes from human samples were almost entirely absent in the case of species with high prevalence in the gut, such as S. thermophilus and Lc. lactis (Fig. 3). Indeed, more than 90% of the S. thermophilus genomes were derived from FFs (mostly dairy products), while higher heterogeneity was observed for Lc. lactis, which was also found in insects, birds, fish and plant material (Fig. 3). Therefore, integrating isolated genomes with MAGs from large-scale metagenomic datasets can help overcome the lack of genomes from human hosts and represents an actual opportunity to advance the field through comparative genomic analyses of LAB, extensively taking into account different populations and environments of origin.

PROBIOTIC TRAITS OF LAB THAT MAKE THEM RELEVANT FOR THE HUMAN GUT AND THEIR PREVALENCE ACROSS GENOMES

Several LAB species are GRAS, due to their centuries-long history of use and human consumption in FFs, and therefore include most of the probiotic species that are currently available on the market. This has boosted the search for and characterization of novel LAB strains with potential applications as probiotics.

Figure 2. Number of LAB reference genomes in NCBI grouped at (A) genera and (B) species level. In (B), only species with at least 10 genomes deposited in NCBI on December 2019 are shown.
Indeed, a Scopus search for ‘probiotic’ and ‘Lactic Acid Bacteria’ returned approximately 4900 documents (December 2019).

The widespread genome sequencing efforts of recent years have led to the availability of hundreds of LAB genomes (Fig. 2), and the new term ‘probiogenomics’ was coined in 2009 (Ventura et al. 2009), describing a discipline aimed at exploring the evolutionary history of commensal and probiotic bacteria and highlighting the genetic bases of their health-promoting activities.

The first desirable feature in a probiotic strain is the ability to survive during passage through the GIT. For the scope of this review, we searched the publicly available genomes of 18 LAB species for 24 genes considered important for the capacity to resist the GIT, adhere to colonic cells, and colonize the intestine and that may be related to the ability of some LAB species commonly found in foods or supplements to reach the GIT and persist in the gut microbiome. A list of the genes and the relevant accession numbers is provided in Supplementary Table S4. Fig. 4 reports the prevalence of these genes in the genomes of 18 LAB species. The genes were predicted using Prokka (Seemann 2014) and were mapped using BlastN against a database containing the genes of interest. A gene was considered present if matched with an identity > 90% over a minimum length > 50%.

EPS production is known to protect microbial strains from acid and bile stress. EPSs are high-molecular-weight sugar polymers secreted by microorganisms into the surrounding environment. According to the chemical composition, two types of EPS, homopolysaccharides (HoPSs) and heteropolysaccharides (HePSs), are synthesized by LAB (Zannini et al. 2016). HoPSs are polymers of glucose or fructose, and depending on the type of molecular linkage, these polymers can be α-glucans, β-glucans, or β-fructans, while HePSs comprise two or more different monosaccharide units, mainly D-glucose, D-galactose, and L-rhamnose. Among food-borne LAB, EPS producers have been described in the genera Streptococcus, Leuconostoc, Lactobacillus, Pediococcus, Oenococcus, Lactobacillus and Weissella (Zannini et al. 2016). In addition to protecting microorganisms from the GIT environment, some EPSs have been reported to exhibit immunomodulatory and anti-inflammatory properties (Castro-Bravo, Wells and Margolles 2018). The EPS gene cluster in LAB may include several glycosyltransferases, polysaccharide
polymerases, a tyrosine kinase (epsC) and its modulator (epsB), a transcriptional regulator (epsA) and a phosphotyrosine phosphatase (epsD), which are differently distributed among different species (Deo, Davray and Kulkarni 2019). These genes are broadly spread in the genomes of the S. thermophilus, Lc. lactis, Lb. casei, and Lb. delbrueckii groups, in which many of them have been identified, but are less frequent in the Lb. reuteri, Lb. brevis, Lb. fermentum, and Lb. curvatus groups (Fig. 4). Several LAB strains with probiotic activities have developed mechanisms to counteract the hostile environment of the GIT, with low pH and the presence of bile acids. Urease activity is one of the mechanisms of defence against acid stress, degrading urea and producing ammonia, which increase the pH in the environment surrounding the microbial cell (Mora and Arioli 2014). Urease was present in the genomes of almost all S. thermophilus strains sequenced but was not detected in public genomes of other LAB species, except for approximately 47% of Lb. reuteri genomes (Fig. 4). In contrast, bile salt hydrolase (bsh) is present in > 90% of the genomes from Lb. reuteri and species within the Lb. plantarum and Lb. delbrueckii groups (Fig. 4). Bile salt hydrolase activity is considered desirable in probiotic strains since it allows the hydrolysis of conjugated bile salts, increasing the possibility of survival of the strain in the GIT (Begley et al. 2006).

Although several probiotic strains can exert a positive health effect without colonization of the GIT, adhesion to intestinal epithelial cells is one of the most commonly screened characteristics during preliminary probiotic characterization, as this property is essential for the competition of the probiotic strain with pathogens for resources and space (Papadimitriou et al. 2015). Colonic epithelial cells are covered by a layer of mucin, a large glycoprotein. Binding to colonic mucin by probiotic bacteria is achieved via a mucus-binding protein (Mub). In addition, the ability of bacterial cells to self-aggregate and form biofilms is considered to influence epithelial adhesion (Papadimitriou et al. 2015; Sanders et al. 2018). The Mub gene was found in 60–90% of the published genomes of Lb. johnsonii, Lb. gasseri, and Lb. acidophilus but was absent in the other LAB species screened, including phylogenetically related species such as Lb. delbrueckii and Lb. helveticus, all belonging to the same taxonomic group. In addition, approximately 94% of the Lb. acidophilus genomes contained a biofilm-associated surface protein. This highlights the high potential of these species to colonize the colonic environment and persist after ingestion. Indeed, these species include most of the commercially available probiotic strains.

Other interesting features that are potentially important for probiotic LAB are the presence of attachment factors, such as fimbriae and pili, and the production of antimicrobial compounds, such as acids, hydrogen peroxide or bacteriocins, which may enhance the ability of the bacteria to compete against other intestinal microbes and could potentially inhibit pathogens (Dobson et al. 2012; Sanders et al. 2019). Bacteriocins are small peptides synthesized ribosomally by a wide range of bacteria and archaea that exert antimicrobial activity against other taxa, such as enterococci and lactococci.
either of the same species as the producer or across genera and against which the producer develops specific immunity-related mechanisms. Bacteriocins are a heterogeneous class of peptides with different structures, sizes, types of activity, immunity-related mechanisms, and target cell receptors (Dobson et al. 2012; Chikindas et al. 2018). More than 90% of the genomes of S. thermophilus, Lb. acidophilus, and Lb. helveticus showed genes involved in bacteriocin production, while these genes were present in approximately 40–50% of Lb. sakei, Lb. plantarum, Lb. johnsonii, and Lc. lactis genomes (Fig. 4). However, the importance of bacteriocin production in probiotic bacteria remains controversial (Dobson et al. 2012). Many bacteriocin-producing microorganisms can inhibit pathogens in vitro (LeBlay et al. 2007), but results regarding in vivo efficacy are scarce and often do not match the in vitro activity. For example, the antimicrobial peptide lacticin 3147 produced by an Lc. lactis strain was effective against Listeria monocytogenes in vitro but failed in a mouse model (Dobson et al. 2011). The same result was reported for a strain of Pediococcus acidilactici: a corresponding effect was not observed in vivo despite its activity in the reduction of L. monocytogenes viability by 3 logs in vitro (Dabour et al. 2009). In contrast, some LAB strains are able to synthesize bacteriocins in vivo, showing direct antagonistic activity against pathogens. For example, Corr et al. (2007) demonstrated that oral gavage of Lb. salivarius UCC118 into mice protected them from L. monocytogenes infection. However, we should note that bacteriocins usually show a limited spectrum of action against target bacteria that are phylogenetically close to the producer. Therefore, bacteriocins produced by LAB are usually active against only Gram-positive bacteria.

HEALTH-RELATED ACTIVITIES OF LAB: EVIDENCE FROM IN VIVO TRIALS

Several studies have reported the health-related functional properties of probiotic LAB. Anti-inflammatory and immunomodulatory effects have been proposed for some LAB strains. For example, a strain of Lb. plantarum was suggested to play an anti-inflammatory role due to a specific structure of its teichoic acids (Granette et al. 2005), while this role was attributed to EPS (Górská et al. 2016), pili (Lebeer et al. 2012) and S-layer proteins (Konstantinov et al. 2008) in strains of Lb. plantarum, Lb. rhamnosus, and Lb. acidophilus, respectively. A strain of Lb. salivarius was able to reduce inflammation and exert a preventive effect on colitis development in mice (Daniel et al. 2006), and the administration of a heat-killed strain of Lb. plantarum ameliorated inflammation and fibrosis in obese rats (Uchinaka et al. 2018). In addition, contact of the growth supernatant of one strain of S. thermophilus with immune cells reduced the release of the inflammatory marker TNF-alpha (Ménard et al. 2004).

The role of LAB in obesity and the related metabolic syndrome remains controversial, and contrasting results have been reported in the literature. The abundance of lactobacilli was found to be higher in obese subjects than in anorexic subjects (Ley et al. 2005) and in type 2 diabetes patients (Larsen et al. 2010; Karlsson et al. 2013). In addition, Drissi et al. (2014) suggested that Lactobacillus species might be differently associated with weight gain or loss; moreover, an enhanced potential for glycolysis, fat digestion, and oxidative stress response in weight gain-associated strains was highlighted when comparing the genomes of the two groups. However, several studies on mouse models have shown that the LAB consumption improved glucose metabolism and hepatic inflammation associated with a high-fat diet (Alard et al. 2016; Park et al. 2017). A similar result was also observed in human clinical trials. Moroti et al. (2012) hypothesized an effect of daily consumption of Lb. acidophilus and B. bifidum strains in reducing glycaemia and cholesterol levels, while Kobyliak et al. (2018b) concluded that supplementation with a multistrain probiotic (containing strains of Lactobacillus, Lactococcus, Bifidobacterium, Acetobacter, Propionibacterium) reduced insulin resistance in type 2 diabetes patients. All these contrasting results highlighted that the role of LAB cannot be generalized and that different species and strains can have a specific effect. Moreover, the overall response is also likely influenced by inter-individual variability.

Most of our knowledge is based on in vitro experiments or animal trials, while the best option to ascertain the possible health benefits of a microbial strain is human randomized controlled trials (Hill et al. 2014; De Filippis et al. 2018a). We surveyed the available literature regarding the effect of dietary intervention with probiotic LAB by searching the Scopus database for documents (only articles written in English) containing the words ‘probiotic’ OR ‘lactic acid bacteria’ AND ‘clinical trial’ OR ‘intervention’ OR ‘treatment’ in the abstract, title or keywords. Animal trials were excluded, as well as review articles and studies in which probiotic strains not belonging to the LAB group were exclusively tested. This search identified a total of 95 studies, which are reviewed and reported in Table 1. Probiotic consumption has been extensively tested in the literature in either healthy or diseased populations (Table 1). Most of the studies used multistrain probiotic products, containing 1 to 10 different strains, with high heterogeneity in the amount ingested, ranging mostly from approximately 10^7 to 10^11 cells/day, in single or multiple doses. When multistain formulations were used, LAB were often administered together with Bifidobacterium spp. strains. In addition, mixed preparations of probiotic strains and prebiotic fibre are often used (sybiotic). Supplementation with probiotic LAB has been proposed for the treatment or improvement of symptoms of several types of diseases, including inflammatory bowel diseases, allergies and intolerance, diabetes and metabolic syndrome, stress and mental disorders, and infant colic. It is important to note that several studies did not report the name of the specific strain(s) used, the viable counts and the number of cells ingested, making comparison across studies impossible. Indeed, many of the probiotic activities were strain specific. For example, two different strains of Lb. acidophilus (LA-5 and NCFM) were tested at a similar dose for a possible role against allergic diseases (asthma and atopic dermatitis, respectively), and only Lb. acidophilus LA-5 was shown to be effective (Table 1).

In some cases, independent clinical trials tested the efficacy of the same probiotic formulation targeting an identical health outcome (Table 1). For example, Chahwan et al. (2019), Steenberg et al. (2015) and colleagues administered a multistrain probiotic preparation (Ecologix® Barrier) containing 4 strains of Lactobacillus spp., 2 strains of Lc. lactis, and 3 strains of Bifidobacterium spp. to adults with depression, and both studies reported an improvement in neurocognitive functions and relief from the symptoms of depression. In contrast, the same formulation was not effective at improving blood glycaemia in type 2 diabetes patients (Horvath et al. 2019). This highlights that a probiotic formulation should be designed to target a well-defined health outcome or population. In two other trials, the consumption of Lb. johnsonii LA1 for 12 or 24 weeks was proposed to reduce the recurrence of Crohn’s disease after surgery (van Gossum et al.
| Probiotic species/strains | Quantity/microbial loads ingested | Placebo control group | Study design* | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved* | Reference |
|--------------------------|---------------------------------|----------------------|---------------|-----------------------------|-----------------------|---------|-------------------------------|----------------------------------------|----------------------|--------------------------|-----------|
| B. animalis sub. lactis CNCM I-2494, Lb. delbrueckii sub. bulgaricus CNCM I-1632 and CNCM I-1519, S. thermophilus CNCM I-1630, Lc. lactis sub. lactis CNCM I-1631 | Counts and daily amount assumed not reported | 50 | Yes | Double-blind, randomized, placebo-controlled trial | Male (36%), adults (55 ± 11), normal-weight | 2 weeks | None | 16S rRNA gene sequencing | IBS symptoms | No | Le Nevé et al. 2019 |
| Bio-25—B. bifidum, Lb. rhamnosus, Lc. lactis, Lb. casei sub. casei, B. breve, S. thermophilus, B. longum sub. longum, Lb. casei sub. paracasei, Lb. plantarum, B. longum sub. infantis; strains not reported | 2 pills/day, 2.5 × 10^{10} CFU/pill | 8 | No | Not reported | Male (63%), adults (28 ± 2.3), normal-weight | 4 weeks | None | 16S rRNA gene and shotgun sequencing | Increase in Akkermansia, Vagococcus, Enterococcus, Blautia, and Lactococcus | Gut microbiota reconstruction upon antibiotic administration | No | Suez et al. 2018 |
| Dicoflor®—Lb. rhamnosus GG | 2 capsules/day, 6 × 10^{6} CFU/capsule | 45 | No | Not reported | Male (84%), adults (47 ± 8), normal-weight | 8 weeks | None | 16S rRNA gene sequencing | Decrease in Enterobacteriaceae | Inflammatory status in HIV patients | Yes | Arnbjerg et al. 2018 |
| DUOLAC 7–S. thermophilus KCTC 11870BP, Lb. plantarum KCTC 10782BP, Lb. acidophilus KCTC 11906BP, Lb. rhamnosus KCTC 12202BP, B. lactis KCTC 11904BP, B. longum KCTC 12200BP, B. breve KCTC 12201BP | 2 capsules/day, 5 × 10^{3} CFU/capsule | 17 | Yes | Double-blind, randomized, placebo-controlled trial | Male (0%), obese, age not reported | 8 weeks | None | Real-time qPCR for specific targets | Increase in B. breve, B. lactis and Lb. rhamnosus | Endotoxemia in obese subjects | Yes | Lee et al. 2014 |
| Ecologics® 825—Lb. casei W56, Lb. acidophilus W22, Lb. paracasei W20, B. lactis W51, Lb. salivarius W24, Lc. lactis W19, B. lactis W52, Lb. plantarum W62, B. bifidum W23 | 3 g/day, 7.5 × 10^{6} CFU/g | 15 | Yes | Double-blind, randomized, placebo-controlled trial | Male (50%), adults (26 ± 9), BMI not reported | 4 weeks | None | - | Participants behaviour | Yes | Bagga et al. 2019 |
| Ecologics® Barrier—B. bifidum W23, B. lactis W51, B. lactis W52, Lb. acidophilus W37, B. breve W63, Lb. casei W56, Lb. salivarius W24, Lc. lactis W19, Lc. lactis W58 | 4 g/day, 2.510^{9} CFU/g | 34 | Yes | Placebo-controlled trial | Male (38%), adults (36 ± 12), normal-weight | 8 weeks | None | 16S rRNA gene sequencing | None | Depression | Yes | Chahwan et al. 2019 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|-----------------------|---------|-------------------------------|-----------------------------------|--------------------------|---------------------|-----------|
| Ecologic® Barrier—see above | 6 g/day, 2.5 × 10^9 CFU/g | 12 | Yes | Double-blind, randomized, placebo-controlled trial | Male (92%), adults (61 ± 9), obese | 24 weeks | No | 16S rRNA gene sequencing | Increase in Lb. brevis | Glucose metabolism in type-2 diabetes patients | No | Horvath et al. 2019 |
| Ecologic® Barrier—see above | 2 g/day, 2.5 × 10^9 CFU/g | 29 | Yes | Double-blind, randomized, placebo-controlled trial | Male (0%), adults (21 ± 1), normal-weight | 4 weeks | No | None | - | | Yes | Papalini et al. 2018 |
| Ecologic® Barrier (see above) | 2 g/day, 2.5 × 10^9 CFU/g | 20 | Yes | Placebo-controlled trial | Male (25%), adults (20 ± 7), normal-weight | 4 weeks | No | None | - | Depression | Yes | Steemersen et al. 2015 |
| Ecologic® Barrier (see above) | 1 serving/day, 2.5 × 10^9 or 1 × 10^10 CFU/serving | 30 | Yes | Double-blind, randomized, placebo-controlled trial | Male (0%), adults (56 ± 7), obese | 12 weeks | No | None | - | Iron metabolism in post-menopause | Yes | Skrypnik et al. 2019 |
| Enterolactis® Plus—Lb. paracasei DG | 2 capsules/day, 2.4 × 10^10 CFU/capsule | 30 | Yes | Double-blind, randomized, placebo-controlled, cross-over trial | Male (48%), adults (35 ± 11), normal-weight | 4 weeks | Yes, 4 weeks between treatments | 16S rRNA gene sequencing | Increase in Proteobacteria and Coprococcus; decrease in Blautia | None | - | Ferrario et al. 2014 |
| Enterolactis® Plus—see above | Counts and daily amount assumed not reported | 20 | Yes | Double-blind, randomized, placebo-controlled, cross-over trial | Male (25%), adults (63 ± 13), BMI not reported | 4 weeks | Yes, 4 weeks between treatments | 16S rRNA gene sequencing | Increase in Lactobacillus and Oscillospira; decrease in Blautia | IBS symptoms | Yes | Cremon et al. 2018 |
| GoodBelly StraightShot—Lb. plantarum Lp299v Heat-killed Lb. gasseri CP2305 | 1 serving/day, 2 × 10^10 CFU/serving | 20 | No | Not reported | Male (100%), adults (63 ± 7), obese | 6 weeks | Yes, 4 weeks | 16S rRNA gene sequencing | Increase in Lactobacillus | Coronary artery disease | Stress and anxiety | Yes | Malik et al. 2018 |
| Heat-killed Lb. paracasei CBA L74 | 1 serving/day, 5.9 × 10^11 CFU/serving | 66 | Yes | Double-blind, randomized, placebo-controlled trial | Male (53%), infants (33 ± 9 months) | 12 weeks | No | None | - | Infection disease occurrence in children | Yes | Corsello et al. 2017 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieveda | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|-----------------------------|-----------------------|---------|-------------------------------|-----------------------------------|-----------------------------|--------------------------|------------|
| Heat-killed Lb. paracasei CBA L74 | 1 serving/day, 5.9 × 10^{11} CFU/serving | 10 | Yes | Double-blind, randomized, placebo-controlled trial | Male (80%), children (33 ± 9 months) | 12 weeks | No | 16S rRNA gene sequencing | Increase in Lactobacillus, Faecalibacterium, Oscillospira | None | - | Berni Canani et al. 2017 |
| LacClean Gold-S®—B. bifidum KCTC 12180BP, B. lactis KCTC 11904BP, B. longum KCTC 12000BP, Lb. acidophilus KCTC 11906BP, Lb. rhamnosus KCTC 12028BP, S. thermophilus KCTC 11878BP | 2 capsules/day, 5 × 10^{9} CFU/capsule | 25 | Yes | Double-blind, randomized, placebo-controlled trial | Male (44%), adults (46 ± 14), BMI not reported | 4 weeks | No | Real-time qPCR for specific targets | Increase in B. lactis, Lb. rhamnosus, S. thermophilus | IBS symptoms | Yes | Yoon et al. 2014 |
| Lb. acidophilus LA-5, B. animalis subsp. lactis BB-12 | 1 serving/day, 1 × 10^{9} CFU/serving | 23 | Yes | Double-blind, randomized, placebo-controlled trial | Male (53%), adults (52 ± 7), overweight | 6 weeks | No | None | - | Glucose metabolism in type-2 diabetes patients | Yes | Bordalo Tonucci et al. 2015 |
| Lb. acidophilus LA-5, Lb. rhamnosus GG, B. animalis subsp. lactis BB-12 | 1 capsule/day, 8.75 × 10^{9} CFU/capsule | 16 | Yes | Double-blind, randomized, placebo-controlled three way cross-over trial | Male (50%), adults (43 ± 20), overweight | 1 week | Yes, 2 weeks between treatments | Real-time qPCR and 16S rRNA gene sequencing | Increase in Bifidobacterium | Asthma | Yes | McLoughlin et al. 2019 |
| Lb. acidophilus NCFM OR B. lactis Bi-07 | 1 serving/day, 1 × 10^{10} CFU/serving | 17 | Yes | Double-blind, randomized, placebo-controlled three way cross-over trial | Infants (7–24 months), sex not reported | 8 weeks | No | 16S rRNA gene sequencing | None | Atopic dermatitis | No | Larsen et al. 2011 |
| Lb. acidophilus PBS066, Lb. reuteri PBS072 OR Lb. plantarum PBS067, Lb. rhamnosus LRH020, B. animalis subsp. lactis BLO50 | 1 serving/day, 5 × 10^{9} CFU/serving | 50 | Yes | Double-blind, randomized, placebo-controlled trial | Adults (37 ± 13), sex and BMI not reported | 8 weeks | Yes, 4 weeks | None | - | IBS with constipation | Yes | Mezzasalma et al. 2016 |
| Lb. acidophilus, B. bifidum, B. lactis, B. longum; strains not reported | 1 serving/day, 1.5 × 10^{9} CFU/serving | 27 | Yes | Double-blind, randomized, placebo-controlled trial | Male (48%), adults (53 ± 8), BMI not reported | 24 weeks | No | None | - | Metabolic syndrome in pre-diabetic patients | Yes | Kassaian et al. 2019 |
Table 1. Continued

| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|---------------------------|-----------------------|---------|-------------------------------|--------------------------------------|-------------------------|------------------------|-----------|
| Lb. acidophilus, B. longum; strains not reported | 2 capsules/day; counts not provided | 37 | Yes | Double-blind, randomized, placebo-controlled trial | Male (30%), adults (44 ± 15), normal-weight | 4 weeks | No | Real-time qPCR for specific targets | None | IBS symptoms | Yes | Cui and Hu 2012 |
| Lb. acidophilus, Lb. casei, Lb. lactis, B. bifidum, B. longum, B. infantis; strains not reported | 2 sachets/day, 3 × 10^{10} CFU/sachet | 68 | Yes | Double-blind, randomized, placebo-controlled trial | Male (46%), adults (53 ± 9), normal-weight or overweight | 12 weeks | No | Microbial counts | Increase in Bifidobacterium and Lactobacillus | Glucose metabolism in type-2 diabetes patients | Yes | Firouzi et al. 2017 |
| Lb. acidophilus, Lb. plantarum, Lb. fermentum, Lb. gasseri; strains not reported | 0.5 g/day, 5 × 10^{10} CFU/g | 45 | Yes | Double-blind, randomized, placebo-controlled trial | Male (0%), adults (30 ± 6), normal-weight | 6 weeks | No | None | - | - | No | Nabhani et al. 2018 |
| Lb. casei 01 | 1 capsule/day, 1 × 10^{8} CFU/capsule | 22 | Yes | Double-blind, randomized, placebo-controlled trial | Male (0%), adults (41 ± 13), overweight | 8 weeks | No | None | - | - | Inflammatory status in rheumatoid arthritis | Yes | Vaghef-Mehrabany et al. 2014 |
| Lb. casei Lcr35® | 2 capsules/day, 2 × 10^{8} CFU/capsule | 42 | No | Prospective, randomized, case-controlled study | Male (57%), children (2.3 ± 1.3), normal-weight | 1 week | No | 16S rRNA gene sequencing and plate counts | Increase in Lachnospiraceae, Bacteroides, Ruminococcus and decrease in Enterobacteriaceae, Escherichia, Clostridium | Diarrhea | Yes | Lai et al. 2019 |
| Lb. casei LMG 101/37 P-17 504, Lb. plantarum CECT 4528, L. animalis sub. lactis B11 LMG P-17 502, B. breve Bb8 LMG P-17 501, B. breve B10 LMG P-17 500 | 1 sachet/day, 10 × 10^{8} CFU/sachet | 54 | Yes | Double-blind, randomized, placebo-controlled trial | Male (11%), adults (43 ± 10), normal-weight | 6 weeks | No | 16S rRNA gene sequencing and plate counts | Increase in Bifidobacterium, Staphylococcus and lactic acid bacteria | Concomitant celiac disease and IBS | Yes | Francavilla et al. 2019 |
| Lb. casei W56, Lc. lactis W19, Lb. acidophilus W22, B. lactis W22, Lb. paracasei W20, Lb. plantarum W62, B. lactis W51, B. bifidum W23, Lb. salivarius W24 | 1 sachet/day; counts not reported | 20 | No | Not reported | Male (55%), adults (77 ± 10), BMI not reported | 4 weeks | No | Real-time qPCR for specific targets | Increase in Faecalibacterium prausnitzii | Alzheimer's disease | Yes | Leblhuber et al. 2018 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|-----------------------------|------------------------|----------|-------------------------------|---------------------------------|-----------------------------|------------------------|-----------|
| Lb. casei Zhang          | 1 tablet/day, 1 × 10^10 CFU/tablet| 24           | No                    | Open-label   | Male (46%), adults (43 ± 17), normal-weight | 4 weeks                | No       | 16S rRNA gene sequencing       | Positive correlation of Lb. casei with Prevotella, Faecalibacterium, Bifidobacterium | None | -                      | Zhang et al. 2014 |
| Lb. casei, Lb. acidophilus, Lb. delbrueckii subsp. bulgaricus, B. breve, B. longum, S. thermophilus; strains not reported | 1 capsule/day, 1.5 × 10^10 CFU/capsule | 30           | Yes                   | Double-blind, randomized, controlled trial | Male (7%), adults (42 ± 3), normal-weight | 8 weeks                | No       | None                          | -                               | Thyroid function | Yes                    | Talebi et al. 2020 |
| Lb. casei, Lb. rhamnosus, S. thermophilus, B. breve, Lb. acidophilus, B. longum, S. thermophilus; strains not reported | 1 serving/day, counts not reported | 25           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (60%), infants (1.5 ± 0.8 months) | 4 weeks                | No       | None                          | -                               | Crying time in infants with colic | Yes                    | Kianifard et al. 2019 |
| Lb. casei, Lb. rhamnosus, S. thermophilus, B. breve, Lb. acidophilus, B. longum, Lb. delbrueckii sub. bulgaricus; strains not reported | 2 capsules/day, 2 × 10^8 CFU/capsule | 20           | Yes                   | Parallel, triple-blind, randomized, controlled trial | Adults (57 ± 1.9), overweight/obese, sex not reported, Male (45%), children (11 ± 2), obese | 12 weeks               | No       | None                          | -                               | Metabolic syndrome | Yes                    | Rabiei et al. 2019 |
| Lb. casei, Lb. rhamnosus, S. thermophilus, B. breve, Lb. acidophilus, B. longum, Lb. bulgaricus; strains not reported | 1 capsule/day, 2 × 10^8 CFU/capsule | 29           | Yes                   | Randomized, triple-masked controlled trial | None | 4 weeks                | No       | Microbial counts              | None                            | Obesity | No                     | Kelishadi et al. 2014 |
| Lb. gasseri CECT5714, Lb. corynformis CECT5711 | 1 serving/day, 2 × 10^9 CFU/serving | 15           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (50%), adults (33 ± 10), BMI not reported | 4 weeks                | Yes, 2 weeks | None                          | -                               | Bowel habits | Yes                    | Olivares et al. 2006 |
| Lb. gasseri KS-13, B. bifidum GS-1, B. longum MM2 | 2 capsules/day, 1.5 × 10^9 CFU/capsule | 16           | Yes                   | Double-blind, randomized, placebo-controlled, cross-over trial | Adults (70 ± 1), sex and BMI not reported | 3 weeks                | Yes, 5 weeks between treatments | None                          | Real-time qPCR for specific targets and 16S rRNA gene sequencing | None | None | Spaisier et al. 2015 |
| Lb. helveticus Bar13, B. longum Bar33 | 1 serving/day, 1 × 10^8 CFU/serving | 16           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (41%), adults (76 ± 10), BMI not reported | 4 weeks | None | Phylogenetic microarray | Increase in Faecalibacterium prausnitzii | None | None | Rampelli et al. 2013 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|-----------------------------------|--------------|-----------------------|--------------|----------------------------|-----------------------|---------|-----------------------------|-------------------------------------|-------------------------|----------------------|----------|
| Lb. helveticus R0052     | 1 sachet/day, 3 x 10^9 CFU/sachet  | 23           | Yes                   | Randomized, double-blind, placebo-controlled trial | Infants (3–12 months), sex and BMI not reported male (29%), adults (36 + 8), normal-weight male (54%), adults (32 + 10), BMI not reported | 8 weeks | No | 16S rRNA gene sequencing | Increase in Proteobacteria, decrease in Firmicutes | - | None | De Andrés et al. 2018 |
| Lb. helveticus R0052, B. longum R0175 | 1 sachet/day, 10 x 10^9 CFU/sachet | 28           | Yes                   | Double-blind, randomized controlled trial | Male (52%), adults (39 + 15), BMI not reported | 8 weeks | No | None | - | Depression | Yes | Kazemi et al. 2019 |
| Lb. johnsonii LA1         | 2 sachets/day, 2 x 10^9 CFU/sachet | 48           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (56%), adults (39 + 15), BMI not reported | 24 weeks | No | None | - | Recurrence of Chron’s disease after surgery | Yes | Marteau et al. 2006 |
| Lb. johnsonii LA1         | 1 sachet/day, 1 x 10^10 CFU/sachet | 27           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (56%), adults (39 + 15), BMI not reported | 12 weeks | No | None | - | - | No | Van Gossum et al. 2007 |
| Lb. plantarum ATTC 202 195 | 1 capsule/day, 1 x 10^9 OR 1 x 10^10 CFU/capsule | 21           | Yes                   | Three arms, randomized, controlled, crossover study | Male (52%), adults (27 + 7), normal-weight | 3 days | No | None | - | Energy intake | No | Toksvig Bjerg et al. 2014 |
| Lb. plantarum CCFM8610    | 1 capsule/day, 1 x 10^9 CFU/capsule | 2185         | Yes                   | Double-blind, randomized, placebo-controlled trial | Infants (0–2 months), sex not reported | 8 weeks | No | None | - | - | Yes | Panigrahi et al. 2017 |
| Lb. plantarum EGCc 13 110 402 | 1 serving/day, 1 x 10^9 CFU/serving | 29           | Yes                   | Placebo-controlled trial | Male (60 or 38%), adults (48 + 15 or 53 + 14), normal-weight | 8 weeks | No | 16S rRNA and groEL gene sequencing | Increase in microbial diversity, Bacteroidetes and B. pseudocatenulatum, decrease in Firmicutes/Bacteroidetes ratio | - | Atopic dermatitis | Yes | Fang et al. 2019 |
| Lb. plantarum Lp115       | 2 capsules/day, 2 x 10^9 CFU/sachet | 23           | Yes                   | Prospective, randomized, placebo-controlled, parallel-group study | Male (22%), adults (52 + 11), over-weight | 12 weeks | Yes, 4 weeks | 16S rRNA gene sequencing | None | Blood lipids | Yes | Costabile et al. 2017 |
| Lb. plantarum Lp115       | 80 ml/day, 1.25 x 10^9 UFC/ml | 12           | Yes                   | Not reported | Male (6%), adults (62 + 4), over-weight | 12 weeks | No | None | - | Metabolic syndrome in post-menopausal | Yes | Barreto et al. 2014 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Length of intervention | Washout | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|-----------------------|---------|-------------------------------|-------------------------------------|-------------------------|-----------------------|-----------|
| *Lb. plantarum* MF1298   | 1 capsule/day, 1 × 10^10 CFU/capsule | 16           | Yes                   | Double-blind, randomized, placebo-controlled trial | 16 weeks | No | None | - | IBS symptoms | No | Farup et al. 2012 |
| *Lb. plantarum* PS128 (DSM 28 632) | 1 capsule/day, 3 × 10^10 CFU/capsule | 36           | Yes                   | Double-blind, randomized, placebo-controlled trial | 4 weeks | No | None | - | Autism spectrum disorder | Yes | Liu et al. 2019 |
| *Lb. reuteri* ATTC PTA 6475 | 2 servings/day, 5 × 10^9 CFU/serving | 45           | Yes                   | Double-blind, randomized, placebo-controlled trial | 48 weeks | No | None | - | Bone loss in old women | Yes | Nilsson et al. 2018 |
| *Lb. reuteri* DSM 17 938 | 5 drops/day, 0.2 × 10^9 CFU/drop | 14           | Yes                   | Double-blind, randomized, placebo-controlled trial | 4 weeks | No | None | - | Crying time in infants with colic | No | Nation et al. 2017 |
| *Lb. reuteri* DSM 17 938 | 5 drops/day, 0.2 × 10^9 CFU/drop | 25           | Yes                   | Double-blind, randomized, placebo-controlled trial | 3 weeks | No | FISH for specific targets | None | Crying time in infants with colic | Yes | Savino et al. 2010 |
| *Lb. reuteri* DSM 17 938 | 5 drops/day, 0.2 × 10^9 CFU/drop | 32           | Yes                   | Double-blind, randomized, placebo-controlled trial | 4 weeks | No | Real-time qPCR for specific targets | None | Crying time in infants with colic | Yes | Savino et al. 2018 |
| *Lb. reuteri* DSM 17 938 | 5 drops/day, 0.2 × 10^9 CFU/drop | 85           | Yes                   | Double-blind, randomized, placebo-controlled trial | 4 weeks | No | None | - | Crying time in infants with colic | No | Sung et al. 2014 |
| *Lb. reuteri* DSM 17 938 | 1 serving/day, 1 × 10^9 CFU/serving | 15           | Yes                   | Double-blind, randomized, placebo-controlled trial | 3 weeks | No | 16S rRNA gene sequencing | None | Crying time in infants with colic | No | Roos et al. 2013 |
| *Lb. reuteri* DSM 17 938 | 1 tablet/day, 1 × 10^9 CFU/tablet | 15           | Yes                   | Double-blind, randomized, placebo-controlled, cross-over trial | 24 weeks | No | 16S rRNA gene sequencing | Increase in microbial diversity and in Firmicutes | None | del Campo et al. 2014 |
Table 1. Continued

| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|--------------------------|----------------------------------|--------------|-----------------------|--------------|----------------------------|------------------------|----------|-------------------------------|-------------------------------------|------------------------|----------------------|----------|
| Lb. reuteri DSM17938      | 1 serving/day, 10^9 OR 10^10 CFU/serving | 15 OR 14     | Yes                   | Double-blind, randomized, placebo-controlled, parallel-group study | Male (80%), adults (65 ± 9), BMI not reported | 12 weeks | No | 16S rRNA gene sequencing | None | Glucose metabolism in type-2 diabetes patients | No | Mobini et al. 2017 |
| Lb. reuteri NCIMB 30 242  | 2 servings/day, from 3 × 10^9 to 10 × 10^9 CFU/serving | 10           | No                    | Randomized, dose-escalation design trial | Adults, normal-weight to obese; sex and age not reported Male (50%), adults (31 ± 4), normal-weight | 4 weeks | No | 16S rRNA gene sequencing | None | Reduction of cholesterol | Yes | Martoni et al. 2015 |
| Lb. rhamnosus 14E4        | 1 serving/day, 1 × 10^10 CFU/serving | 14           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (47%), infants (1.3 ± 0.4 months) | 2 weeks | Yes, 1 week | 16S rRNA gene sequencing | Increase in Prevotella and decrease in Bacteroides | None | - | Bautista-Gallego et al. 2019 |
| Lb. rhamnosus GG          | 1 serving/day, 4.5 × 10^9 CFU/serving | 15           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (59%), children (7 ± 2), BMI not reported | 4 weeks | No | None | - | Crying time in infants with colic | No | Pártty et al. 2015 |
| Lb. rhamnosus GG          | 1 serving/day, 6 × 10^9 CFU/serving | 10           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (30%), adults (47 ± 6), normal-weight | 4 weeks | No | DGGE and FISH for specific targets | Increase in Bacteroides | Inflammatory status in cystic fibrosis children | Yes | Bruzzese et al. 2014 |
| Lb. rhamnosus GG          | 1 bottle/day, 1.55 × 10^11 CFU/bottle | 10           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (30%), adults (47 ± 6), normal-weight | 3 weeks | No | Phylogenetic microarray | Increase in Lb. rhamnosus | None | - | Lahti et al. 2013 |
| Lb. rhamnosus GG          | 1 serving/day, 1 × 10^9 CFU/serving | 8            | Yes                   | Double-blind, randomized, placebo-controlled trial | Infants (6 months); sex not reported | 24 weeks | No | Phylogenetic microarray | None | None | None | Cox et al. 2010 |
| Lb. rhamnosus GG          | 4.5 × 10^7 to 8.5 × 10^7/g, day amount not reported | 12           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (75%), infants (1–12 months) | 24 weeks | No | 16S rRNA gene sequencing | Increase in Blautia, Roseburia, Coprococcus | Oral tolerance in cow's milk allergic children | Yes | Bemi Canani et al. 2016 |
| Lb. rhamnosus GG, B. animalis sub. lactis BB-12 | 1 capsule/day, 1 × 10^9 CFU/capsule | 207          | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (0%), adults (32 ± 4.8), from normal-weight to obese | 8 weeks | No | None | - | Gestational diabetes | No | Callaway et al. 2019 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design* | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved* | Reference |
|--------------------------|----------------------------------|-------------|-----------------------|--------------|--------------------------|-----------------------|---------|-------------------------------|---------------------------------|--------------------------|--------------------------|------------------------|
| *Lb. rhamnosus GG, Lb. rhamnosus LC705, B. breve Bb99, Propionibacterium freudenreichii subsp. shermanii J6* | 1 capsule/day, 5 × 10^9 CFU/capsule | 461 | Yes | Double-blind, randomized, placebo-controlled trial | Male (50%), children (1.2 ± 0.3) | 24 weeks | No | None | - | Allergic diseases | No | Kukkonen et al. 2007 |
| *Lb. rhamnosus GG, Lb. rhamnosus LC705, Propionibacterium freudenreichii subsp. shermanii J6, B. animalis subsp. lactis Bb12* | 120 ml/day, 1 × 10^7 CFU/ml | 43 | Yes | Double-blind, randomized, placebo-controlled trial | Male (9%), adults (50 ± 13), normal-weight | 20 weeks | No | Phylogenetic microarray | None | IBS symptoms | Yes | Kajander et al. 2007 |
| *Lb. rhamnosus IMC 501, Lb. paracasei IMC 502* | 1 serving/day, 1 × 10^9 CFU/serving | 25 | Yes | Double-blind, randomized, placebo-controlled trial | Adults; sex, age and BMI not reported | 12 weeks | Yes, 2 weeks | Microbial counts | Increase in lactobacilli and bifidobacteria | Bowel habits | Yes | Verdenelli et al. 2011 |
| *Lb. salivarius CECT5713* | 2 capsules/day, 1 × 10^9 CFU/capsule | 20 | Yes | Double-blind, randomized, placebo-controlled trial | Male (50%), adults (33 ± 8), BMI not reported | 4 weeks | No | Microbial counts | Increase in lactobacilli | Immune response | Yes | Sierra et al. 2010 |
| *Lb. salivarius, strain not reported* | 1 serving/day, 2 × 10^10 CFU/serving | 27 | Yes | Double-blind, randomized, placebo-controlled trial | Adults (25 ± 5), normal-weight, sex not reported | 16 weeks | No | None | - | Upper respiratory tract infections occurrence in athletes | None | Gleeson et al. 2012 |
| *Lb. casei; strain not reported* | 1 bottle/day, 6.5 × 10^9 CFU/bottle | 6 | No | Not reported | Male (33%), children (13 ± 3), normal-weight | 6 weeks | No | Intergenic spacer profiling (IS-pro) | None | - | El Manouni et al. 2019 |
| *Leuconostoc holzapfelii; strain not reported* | Not provided | 21 | No | Not reported | Male (43%), adults (28 ± 2.3), normal-weight | 4 weeks | No | 16S rRNA gene sequencing | None | None | - | Yang et al. 2019 |
| *S. thermophilus, Lb. acidophilus, B. longum; strains not reported* | 3 capsules/day, 3 × 10^10 CFU/capsule | 16 | Yes | Double-blind, randomized, placebo-controlled trial | Male (69%), adults (54 ± 11), normal-weight | 12 weeks | No | DGGE | None | Chronic kidney disease | No | Borges et al. 2018 |
| *Supherb Bio-25-B. bifidum, Lb. rhamnosus, Lc. lactis, Lb. casei, B. breve, S. thermophilus, B. longum sub. longum, Lb. paracasei, Lb. plantarum and B. longum sub. infantis; strains not reported* | 2 pills/day, 2.5 × 10^10 CFU/pill | 14 | Yes | Placebo-controlled trial | Male (57%), adults (42 ± 13), normal-weight | 4 weeks | No | Shotgun metagenome, real-time qPCR | Probiotic strains engraftment is dependent on individualized gut microbiome features | None | - | Zmora et al. 2018 |
Table 1. Continued

| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|---------------------------|----------------------------------|--------------|-----------------------|--------------|-----------------------------|------------------------|---------|-------------------------------|----------------------------------------|--------------------------|-------------------------|-----------|
| Symbiter—* Lactobacillus* + *Lactococcus, Bifidobacterium, Propionibacterium, Acetobacter,* species and strains not reported | 10 g/day, $6 \times 10^{10}$ CFU/g | 31 | Yes | Double-blind, randomized, placebo-controlled trial | Adults (52 ± 2), obese, sex not reported | 8 weeks | No | None | - | Insulin resistance in type-2 diabetes patients | Yes | Kobyliak et al. 2018a |
| Symbiter—see above | 10 g/day, $6 \times 10^{10}$ CFU/g | 30 | Yes | Double-blind, randomized, placebo-controlled trial | Adults (53 ± 10), obese, sex not reported | 8 weeks | No | None | - | Non-Alcoholic Fatty Liver Disease | Yes | Kobyliak et al. 2018b |
| 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 | 100 | Yes | Double-blind, randomized, placebo-controlled trial | Male (32%), adults (39 ± 11), BMI not reported | 12 weeks | No | None | - | IBS symptoms | Yes | Sisson et al. 2014 |
| Vivomixx®—*Lb. plantarum DSM 24 730, S. thermophilus DSM 24 731, B. breve DSM 24 732, Lb. paracasei DSM 24 733, Lb. delbrueckii subsp. bulgaricus DSM 24 734, Lb. acidophilus DSM 24 735, B. longum DSM 24 736, B. infantis DSM 24 737* | 2 sachets/day, $4.5 \times 10^{11}$ CFU/sachet | 9 | Yes | Double-blind, randomized, placebo-controlled trial | Male (100%), adults (45 ± 10), BMI not reported | 24 weeks | No | None | - | Neuroinflammation in HIV patients | Yes | Cecarelli et al. 2017 |
| VSL#3®—*S. thermophilus, B. breve, B. longum, B. infantis, Lb. acidophilus, Lb. plantarum, Lb. paracasei, Lb. delbrueckii; strains not reported* | 2 sachets/day, $1.1 \times 10^{11}$ CFU/sachets | 14 | No | Open-label, single-arm study | Male (0%), adults, age and BMI not reported | 4 weeks | Yes, 4 weeks | 16S rRNA gene sequencing | None | Immune response | Yes | Singh et al. 2018 |
| VSL#3®—see above | 1 capsule/day, $1.1 \times 10^{11}$ CFU/capsule | 15 | Yes | Randomized, placebo-controlled trial | Adults (50 ± 10), overweight or obese, sex not reported | 6 weeks | No | Microbial counts | None | Cholesterol in obese subjects | Yes | Rajkumar et al. 2014 |
| VSL#3®—see above | 1 capsule/day, $1.1 \times 10^{11}$ CFU/capsule | 15 | Yes | Double-blind, randomized, placebo-controlled trial | Adults, sex, age and BMI not reported | 8 weeks | No | Phylogenetic microarray | None | Diarrhea-predominant IBS symptoms | Yes | Michail and Kenche 2011 |
| VSL#3®—see above | 2 sachets/day, $9 \times 10^{10}$ CFU/sachets | 10 | No | Open-label | Male (20%), adults (46 ± 10), BMI not reported | 4 weeks | No | 16S rRNA gene sequencing | Decrease in Bacteroides | IBS symptoms | Yes | Ng et al. 2013 |
| Probiotic species/strains | Quantity/microbial loads ingested | Participants | Placebo control group | Study design | Participant characteristics | Length of intervention | Wash out | Gut microbiome analysis method | Variation in gut microbiome detected | Health outcome targeted | Health outcome achieved | Reference |
|---------------------------|----------------------------------|-------------|-----------------------|-------------|-----------------------------|-----------------------|---------|-------------------------------|--------------------------------------|--------------------------|-----------------------|-----------|
| VSL#3—see above           | $1.1 \times 10^{11}$ CFU/sachets; day amount not reported | 20          | No                    | Not reported | Male (65%), adults (44 ± 27), BMI not reported | 4 weeks               | No      | None                          | -                                    | Liver cirrhosis            | Yes                    | Marlicz et al. 2016 |
| VSL#3—see above           | 2 sachets/day, $1.1 \times 10^{11}$ CFU/sachets | 22          | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (45%), children (10 ± 2), obese | 16 weeks              | No      | None                          | -                                    | Non-Alcoholic Fatty Liver Disease in obese children | Yes                    | Alisi et al. 2014 |
| VSL#3—see above           | $1.1 \times 10^{11}$ CFU/sachets; day amount not reported | 9           | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (33%), adolescents (14 ± 2), obese | 16 weeks              | No      | 16S rRNA gene sequencing       | None                                 | Obesity                  | No                    | Jones et al. 2018   |
| VSL#3—see above           | 1 sachet/day, $9 \times 10^{11}$ CFU/sachet | 66          | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (85%), adults (48 ± 9), BMI not reported | 24 weeks              | No      | None                          | -                                    | Recurrence of hepatic encephalopathy in patients with cirrhosis | Yes                    | Dhiman et al. 2014 |
| Yakult—Lb. casei Shirota   | 1 bottle/day, $6.5 \times 10^{9}$ CFU/bottle | 10          | Yes                   | Double-blind, randomized, placebo-controlled trial | Adults: age, sex and BMI not reported | 20 weeks              | No      | None                          | -                                    | Immune response in allergic rhinitis | Yes                    | Ivory et al. 2008   |
| Yakult—Lb. casei Shirota   | 1 bottle/day, $1 \times 10^{11}$ CFU/bottle | 23          | Yes                   | Double-blind, randomized, placebo-controlled trial | Male (52%), adults (23 ± 0.4), normal-weight | 8 weeks               | No      | 16S rRNA gene sequencing       | None                                 | Stress-induced abdominal dysfunction | Yes                    | Kato-Kataoka et al. 2016 |
| Zircombi—B. longum, Lb. rhamnosus HN001 | 1 sachet/day, $4 \times 10^{9}$ CFU/sachet | 23          | Yes                   | Double-blind, cross-over, randomized, placebo-controlled trial | Male (17%), adults (48 ± 3), normal-weight | 4 weeks               | Yes, 2 weeks between treatments | 16S rRNA gene sequencing | Increase in Bifidobacterium, decrease in Enterobacter, Klebsiella, Salmonella | Yes                    | Vitellio et al. 2019 |
| Lb. johnsonii LA1         | 80 ml/day, $1 \times 10^{7}$ CFU/ml | 74          | Yes                   | Double-blind, cross-over, randomized, placebo-controlled trial | Male (57%), children (12 ± 2), BMI not reported | 3 weeks               | No      | None                          | -                                    | Helicobacter pylori infection eradication | Yes                    | Gotteland et al. 2008 |

*As reported in the original study.
2007; Marteau et al. 2006). The investigators considered two different doses (4 × 10^4 or 1 × 10^10 CFU/day), but the treatment was shown to be ineffective in both trials.

In other cases, the results obtained in separate trials were discordant. The same strain of Lb. reuteri (DSM 17 938) was independently examined for its activity against infant colic in five trials, all with similar duration (2–3 weeks) and number of cells ingested per day (10^8 CFU/day). However, only two of the trials reported a reduction in crying time (Savinio et al. 2010, 2018; see Table 1).

Notably, none of the studies presented in this review commented on the rationale for choosing a specific probiotic dosage. The ISAPP provides suggested amounts ranging from 1 × 10^8 to 1.8 × 10^12 CFU once or twice a day, depending on the strain and the type of target population (Guarnier et al. 2012). However, this list covers gastrointestinal disorders only and does not take into account the intrinsic variability in the gut microbiota. In general, different dosages should be assessed to understand the effect of the dose-response relationship of probiotic consumption on targeted health outcomes and support the causality of the observed associations. This might at least partly explain the presence of contrasting results observed in clinical trials found in the literature.

Contrasting results might also be related to a subject-specific response associated with individual characteristics of the gut microbiome. In fact, several studies reported that different individuals may respond differently to the same drug, dietary treatment or even probiotic treatment and that this difference may be at least partially related to the individual structure of the gut microbiome (Zmora et al. 2016; De Filippis et al., 2018). According to some recent studies, the baseline microbiome may also influence the possibility of the probiotic strain colonizing the gut and its long-term engraftment and persistence once oral administration is stopped (Maldonado-Gomez et al. 2016; Zmora et al. 2018). Nevertheless, most of the studies found in the literature did not explore the effect of probiotic administration on the gut microbiota composition (Table 1). Moreover, even when this analysis was included, the method used did not allow tracking of the fate of the specific strain except in a few cases (Suez et al. 2018; Zmora et al. 2018). Indeed, probiotic treatment was frequently not able to modify the overall structure of the gut microbiota, and only a few studies reported an increase in the abundance of the microbial genus/genera administered. Finally, interpretation of the effect of probiotic consumption on the composition of the gut microbiota may be particularly complicated due to the lack of consensus around a universally accepted definition of a healthy gut microbiota (Bäckhed et al. 2012; Cani 2018; McBurney et al. 2019).

As reported above, some LAB strains may exhibit health-promoting activity even if inactivated. Some clinical trials demonstrated that the consumption of fermented matrices in which probiotic bacteria were heat-inactivated may still have a positive effect on health (Table 1). For example, consumption of milk fermented by Lb. paracasei CBA L74 and subsequently heat-killed reduced the occurrence of infectious diseases in children and boosted the production of beneficial short-chain fatty acids (Berni Canani et al. 2017; Corsello et al. 2017). In another trial conducted in an adult population, the consumption of inactivated Lb. gasseri CP2305 in tablets reduced anxiety and stress (Nishida et al. 2019).

FERMENTED FOODS AND HUMAN HEALTH

Growing evidence has been provided in the literature regarding the enhanced functional and nutritional properties of FFs. Notably, a large proportion of such foods contain living microorganisms, including LAB, which are genetically similar to the strains used as probiotics. Extensive clinical trials have been conducted to prove the human health-promoting activities of probiotics, as reviewed in the previous section. Epidemiological and clinical studies on FFs have also been similarly conducted, although there are considerably fewer such studies, and a large fraction of them have been well reviewed in (Marco et al. 2017; Fernandez and Marette 2018; Kok and Hutkins 2018).

Consumption of fermented dairy products was invariably correlated with overall mortality (Soedamah-Muthu et al. 2013) and impaired glucose metabolism (Eussen et al. 2016) and linked to overall improvements in health-linked biomarkers (González et al. 2019). Yogurt consumption was inversely associated with type 2 diabetes (Chen et al. 2014; Díaz-López et al. 2016), risks of metabolic syndrome (Babio et al. 2015), risk of colorectal cancer (Pala et al. 2011), and long-term weight gain (Mozaffarian et al. 2011). Multiple randomized, controlled trials showed a higher effectiveness of probiotic yogurts than conventional yogurts in the improving various health outcomes, such as fasting blood glucose levels (Ejtaheh et al. 2012) and insulin resistance (Asemi et al. 2013; Nabavi et al. 2015). Different species and strain types present in fermented milk were related to reduced muscle soreness (Iwasa et al. 2013), improved intrinsic brain activity (Tillisch et al. 2013), reduced incidence of fever (Nagata et al. 2016), improved bowel movements (Nagata et al. 2016), reduced risk of cardiovascular disease (Sonestedt et al. 2011), beneficial effects on metabolism (Fernandez and Marette 2018), and positive changes in health and mood (Baars 2019). High consumption of cultured milk lowered the risk of developing bladder cancer (Larsson et al. 2008). Combined consumption of cheese, yogurt, and fermented milk was inversely associated with diabetes (Slujs et al. 2012). Consumption of fermented kimchi decreased insulin resistance, increased insulin sensitivity (An et al. 2013) and reduced the prevalence of asthma and atopic dermatitis (Park and Bae 2016; Kim, Ju and Park 2017). In a randomized controlled study, kimchi also improved fasting blood glucose levels and other health outcomes (Kim et al. 2011; Choi et al. 2013). Kefr-fermented milk was associated with a large six-month increase in hip bone mineral density among osteoporotic patients (Tu et al. 2015), and Chungkookjang improved multiple parameters associated with obesity (Byun et al. 2016). The risk of high blood pressure was reduced by the consumption of fermented products such as miso and natto (Nozue et al. 2011). Total cholesterol levels were improved by consumption of fermented soy (Cavallini et al. 2016) and Kochujang (Lim et al. 2015). Signs of irritable bowel syndrome were attenuated by consumption of low-FODMAP rye bread (Laatikainen et al. 2016) and lacto-fermented sauerkraut (Nielsen et al. 2018). Clinical trials on FFs have also been conducted in animal models. Examples in mice are represented by the alleviation of atopic dermatitis through cream cheese (Kim, Kim and Kim 2019) and the recovery from antibiotic-induced gut dysbiosis with gut barrier function enhancement through a mixture of Lactobacillus species isolated from traditional FFs (Shi et al. 2018). Focusing specifically on the impact of FFs on gastrointestinal health, there is still limited evidence of the effectiveness of these foods (Mota de Carvalho et al.
CONCLUSIONS

FFs are widespread worldwide and can be considered as primary reservoirs of live bacteria that can potentially reach the gut microbiome and eventually impact host health. Although many speculations about the food-gut axis exist, there is only limited evidence supporting a direct effect of FF consumption on the gut microbiome and on the possible transfer of LAB from FFs to the host gut microbiome. Well-designed clinical trials and broad population studies are necessary to understand whether ingested LAB are effectively able to engrain in the human gut and become permanent members of the gut microbiome. As discussed, the ability of certain strains to reach and colonize the human gut will strongly depend on their genomic capacity to counteract the barriers that they will face (i.e. low pH and bile salts), as well as the specific composition of the host gut microbiome. In future years, probiotic research will surely benefit from recent advances in genomics and metagenomics and the development of new bioinformatic algorithms and analysis tools. To develop a robust knowledge of the LAB-food-gut axis, comparative genomic studies will be needed to compare the diversity and functions of LAB from food and gutenvironments. In addition, improved availability of LAB genomes from gut isolates will also be important for better understanding the genomic features (if any) that can be pivotal for the adaptation of LAB to the gut environment. Meanwhile, FFs will continue to serve as inexhaustible sources of potential probiotic LAB strains and as natural dietary sources of live LAB cells with a promising role in human gut health.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

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