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A Genomic Perspective on Niche Adaptability in *Lactobacillus*

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Abstract

The *Lactobacillus* genus comprises more than 200 formally recognized species characterized by a phylogenetic and metabolic diversity that exceeds that of a typical bacterial family. The widespread dissemination of members of the lactobacilli in different ecological niches testifies to their extraordinary niche adaptability. Advances in sequencing technologies have facilitated a comprehensive examination of the characteristics of the *Lactobacillus* genus through large-scale comparative genomics, and aided an understanding of the genomic background underpinning the presence of lactobacilli in such a broad range of habitats. Comparative genomic analysis has revealed that adaptation to such highly variable environments is a result of genome evolution. Gene loss and acquisition, mainly driven by horizontal gene transfer, underlies the remarkable genomic diversity observed, resulting in species which may be considered either niche generalists or niche specialists. Larger genome sizes are associated with ecologically flexible species such as *Lactobacillus casei* and *Lactobacillus plantarum.* These niche generalists have typically acquired or retained the capacity to migrate between different habitats and have recently been described as nomadic. Niche specialists, or host-adapted species such as *Lactobacillus sanfranciscensis*, possess much smaller genomes, reflecting ecological specialization. For many species, sufficient information to infer their real niche preferences remains elusive. In this chapter, we review the available genomic information for the *Lactobacillus* genus and the comparative genomic approaches that have been taken to evaluate strains or species found in different niches, which give an insight into niche adaptation within the genus.

Introduction

An ecological niche can be described as a multidimensional space of resources and environmental conditions that together define where an organism can survive and grow. Bacteria generally share niche and resources with other microbial species as well as more complex organisms, and thus they have evolved mechanisms to communicate, to compete and to adapt to environments as diverse as humans, animals or plants (Aussel *et al*., 2016). The potential of a bacterial species to exploit a particular niche is dictated by factors such as environmental conditions, nutrient availability and the presence of competitors, predators or bacteriophages (Pereira and Berry, 2017). Evolutionary adaptation to diverse nutritional niches differs fundamentally in bacteria when compared to higher organisms. Bacteria adapt by remodelling their genomes. Expansion and contraction in gene content is driven by horizontal modes of gene transfer, mechanisms that play such a frequent and profound role in the evolution of bacterial genomes that they often mask vertical patterns of descent. Indeed, genome comparisons of many different bacterial species from a range of ecological niches has revealed a prominent role for gene gain and loss in the processes of niche adaptation,

ecological specialization, host-switching, and other lifestyle changes (Didelot *et al.*, 2009). However, microbial species can often also grow in a range of conditions much broader than its actual niche. This generalism is widely observed in nature and it is generally accepted that heterogeneity of the natural environments in which organisms grow promotes phenotypic variations giving rise to certain ecological advantages (Woodcock *et al.*, 2017).

The lactic acid bacteria (LAB) are a diverse group of Gram-positive obligately fermentative microorganisms that produce lactic acid as the main product of sugar degradation (for review, see (McAuliffe, 2017)). This group of organisms is currently classified in the order Lactobacillales and comprises species of the genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Oenococcus* and *Pediococcus* among others. While members of the LAB group are closely related phylogenetically, they occupy a diverse set of ecological niches from plants and fermenting plant material to milk to the gastrointestinal tract and genitourinary tract of animal and humans. Such niche diversity among closely related species suggests considerable genetic adaptation during their evolution. Indeed, comparative and functional genome analysis of multiple LAB species and strains has revealed a central trend in LAB evolution: the loss of ancestral genes and metabolic simplification towards adaptation to nutritionally rich environments (Makarova and Koonin, 2007).

The *Lactobacillus* genus is a broadly defined group within the LAB that are closely associated with terrestrial and marine animals, their environment (plants, materials of plant origin, manure) and their food (cheese, yoghurt) (Sun *et al.*, 2015). It represents the largest and most diverse of the LAB genera, comprising some 200 formally recognized species with applications in industrial, biotechnological and medical fields. This heterogeneous group occupies niches that are largely variable in conditions such as nutrient availability, temperature and the presence of other (competing) bacteria. Nonetheless, lactobacilli are able to adapt to these specific conditions and successfully colonize these varying habitats. This adaptability originates from the remarkable genetic diversity of this genus that is much more extensive than that normally observed at the family level (Salvetti and O'Toole, 2017). Application of high-throughput whole genome sequencing and the associated 'omics' technologies have completely revolutionized the study of this important group of organisms, from providing a comprehensive view of the remarkable diversity within the group through to the development of improved or novel strains and their associated processes in industrial application. In this chapter, we provide an overview of the developments in *Lactobacillus* research during this genomic era, with particular emphasis on the genetic events responsible for the adaptation of these organisms to their specific niches.

Genomics of *Lactobacillus* and insights into niche adaptation

The availability of genome sequences has facilitated a deeper examination of the characteristics of the *Lactobacillus* genus, and enabled an understanding of the genomic background for presence of lactobacilli in such a broad range of habitats. Firstly, the genomes of lactobacilli vary in size between 1.23 Mbp (*L. sanfranciscensis*) up to 4.91 Mbp (*L. parakefiri*) (Sun *et al.*, 2015). Based on the analysis of 213 genomes of lactobacilli and associated genera of LAB, the *Lactobacillus* pangenome, consisting of all genes present in a given set of genomes, was estimated to contain 45,000 gene families, while 73 genes mainly responsible for cell growth and replication make up the core genome, shared by all of the analysed strains (Sun *et al.*, 2015). The comparative genome analysis of lactobacilli confirmed the overall trend observed in other LAB, which is minimization of genomes through loss of ancestral genes, but also acquisition of genes by horizontal gene transfer, as a response to adaptation to a specific habitat of these bacteria (Makarova *et al.*, 2006). Furthermore, an increasing number of *Lactobacillus* sequences revealed the potential link between genomic characteristics and specific environmental niche inhabited. To better understand the connection between the habitat and the genomic content, the focus of genomic studies has moved from the comparison of genomes of a single species or groups of related species to metagenomic analysis of isolates present in a niche. These studies have shown that larger genome sizes characterize species able to survive in niches with varying environmental conditions, while species with smaller genomes are adapted for highly specific environments and

less variable conditions, thus leading to simplification of metabolic repertoire, a higher occurrence of pseudogenes and lower GC content (Papizadeh *et al.*, 2017). Both of these trends were confirmed in the genomes of lactobacilli, where larger genomes of lactobacilli corresponded to the free-living and nomadic species, while in host-specialized species, both genome size and GC content were significantly smaller (Fig. 1.1) (Duar *et al.*, 2017b). Higher numbers of genes in free-living organisms are necessary for their survival in habitats differing in nutrients, temperature and the presence of other competing bacteria. On the other hand, a transition to nutritionally rich environments [dairy products and other fermented foods or the gastrointestinal tract (GIT)] has resulted in metabolic simplification and the loss of the ability for biosynthesis of many cofactors, vitamins and amino acids (Papizadeh *et al.*, 2017). In the following sections, characteristics defining adaptation to various niches from which lactobacilli are isolated are discussed.

The dairy niche

In an evolutionary sense, the dairy niche represents a recently occurring man-made niche (Douillard and de Vos, 2014). As such, it does not strictly represent a 'natural' niche for lactobacilli (Duar *et al.*, 2017b) as lactobacilli have been essentially 'domesticated' for use in food and feed production.

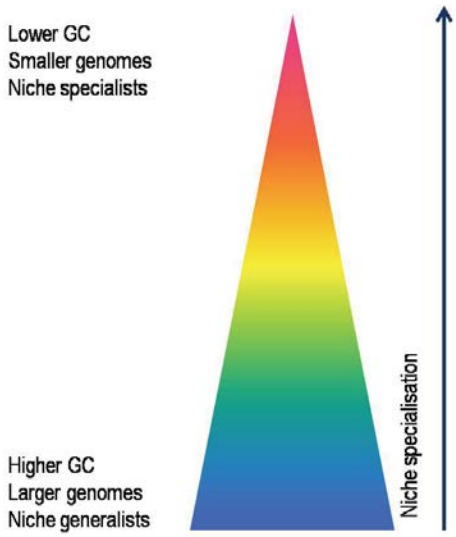

Figure 1.1 Correlation of genome composition to ecological specialization in the *Lactobacillus* genus.

However, genomic studies of species regularly isolated from dairy products have revealed specific characteristics of dairy isolates which cannot be overlooked, as they demonstrate some proof of niche specialization events. Dairy specialization is a common characteristic of several species that are not exclusively isolated from dairy products, and it unavoidably occurred through continuous propagation of these strains in milk fermentations. Numerous comparative genomic studies of species isolated from the dairy niche, among others, reveal several important genomic characteristics of dairy isolates. In these strains, a very limited spectrum of carbohydrate utilization genes is present, due to the fact that in the milk environment, lactose is the dominant sugar. A comparative study of *Lactobacillus casei* strains showed distinct differences in the metabolic characteristics of nine strains isolated from various sources, where dairy isolates were able to utilize the most limited carbohydrate profile, compared to plant or mammalian isolates (Broadbent *et al.*, 2012). Among *Lactobacillus paracasei* genomes, it was shown that strains of dairy origin possess lower numbers of phosphotransferase system (PTS) cassettes which are involved in carbohydrate utilization (Smokvina *et al.*, 2013). Similar findings were confirmed for the dairy adapted species *Lactobacillus delbrueckii* subsp. *bulgaricus*, commonly used in yoghurt production. It was shown for this species that, in addition to a limited number of genes for sugar utilization, a high number of pseudogenes were present (van de Guchte *et al.*, 2006). Genome analysis of the dairy isolate *Lactobacillus helveticus* DPC4571 revealed that this strain lacked genes of the PTS system, cell wall anchoring proteins and mucus-binding proteins, all of which would be redundant in the dairy niche (Callanan *et al.*, 2008). In a comparative study that analysed 100 *Lactobacillus rhamnosus* strains, most of the dairy isolates grouped together, and were characterized by the presence of genes encoding lactose, maltose and rhamnose metabolism and the absence of pili, antimicrobial resistance genes, stress resistance genes and other functions that provide the adaptability to various range of habitats (Douillard *et al.*, 2013). All of these studies suggested that adaptation to the nutritionally stable and rich dairy niche facilitates loss of superfluous genes, as there is no requirement to maintain uptake systems for a variety of substrates.

Another phenomenon that contributes to nutrient-rich habitat adaptation of lactobacilli is the acquisition of new genes and an increase in number of paralogous genes. These can emerge in specific gene duplication events or through horizontal gene transfer (HGT) (Makarova and Koonin, 2007). For instance, a genomic island with different GC content compared to the rest of the genome was detected in genome of *L. helveticus* DPC4571, and included genes encoding enzymes of fatty-acid and amino acid metabolism (Callanan *et al.*, 2008). Similarly, the multiple peptidases or proteases encoded in the genomes of dairy *L. helveticus*, which are thought to be the result of gene acquisition events, along with the decay of the genes associated with survival in the GIT, explain the high affinity of these strains to the cheese environment as a protein-rich niche. The presence of a higher number of proteinases with different but complimentary substrate and cleavage specificities could explain the efficiency of the *L. helveticus* proteolytic system which provides an adaptive advantage regarding milk protein hydrolysis (Genay *et al.*, 2009). The variability in proteinase gene content could be seen as an important industrial feature, contributing to the desired flavour development (Broadbent *et al.*, 2011).

Other food-related niches

The meat environment

Certain *Lactobacillus* species are part of the indigenous microbiota of meat and may also be used in the fermentation and preservation of meat products. Meat is a rich source of peptides and amino acids, but a relatively poor source of sugars. An example of a species which has demonstrated specific metabolic adaptations to life in the meat environment is *Lactobacillus sakei* (Nyquist *et al.*, 2011). In response to the presence of meat proteins, *L. sakei* has been shown to up-regulate genes encoding oligopeptide transporters and intracellular peptidases to take advantage of these substrates (Fadda *et al.*, 2010; Xu *et al.*, 2015). The capacity to metabolize certain amino acids that are abundant in meat is also a feature of this species. *L. sakei* use arginine, via the arginine deiminase pathway (ADI), to generate ornithine, ammonia, CO_2 and ATP (Rimaux *et al.*, 2012) (Fig. 1.2). The capacity to metabolize arginine is thought to enhance the survival of *L. sakei* in the meat environment as a result of the generation of ATP. This pathway is induced by the presence of arginine and by anaerobiosis, conditions common during meat storage. *L. sakei* is one of the best equipped of the *Lactobacillus* species to cope with growth under oxidative stress

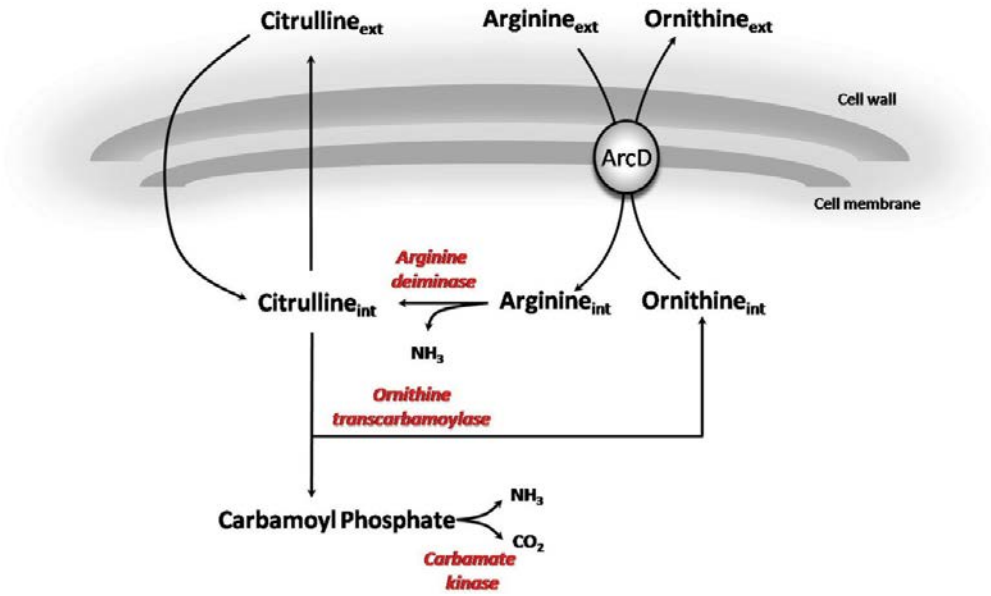

Figure 1.2 Catabolic pathway for arginine in *Lactobacillus sakei* through the arginine deiminase (ADI) pathway. Enzymes are indicated in red. ArcD represents arginine/ornithine antiporter pathway.

as occurs with anaerobiosis, despite the fact that it is a facultative anaerobe and its main metabolism is fermentation. It has been proposed that the presence of a mutated cytochrome P450 gene in some *L. sakei* strains may enable them capable of some form of respiration (Nyquist *et al.*, 2011).

As previously mentioned, meat is a poor source of sugars as carbon sources. One of the sugars present in meat is ribose, and *L. sakei* has been shown to be capable of using ribose through an ATP-dependent system. However, unlike other species, ribose uptake in *L. sakei* is not via ATP-binding cassette (ABC) transporters but via a ribose transporter suspected to function as a facilitator encoded by the *rbsU* gene (McLeod *et al.*, 2011). Nucleosides are also an important carbon source for this species, as evidenced by the redundancy of genes involved in exogenous nucleoside scavenging in the *L. sakei* 23K genome (Nyquist *et al.*, 2011). The ability of *L. sakei* 23K to use N-acetylneuraminic acid (NANA) as a carbon source has also been documented (Anba-Mondoloni *et al.*, 2013). A pathway (*nanTEAR–nanK*) encoding transport and the early steps of catabolism of this amino sugar, which is present in meat, has been identified in *L. sakei* 23K but absent from the genomes of other *L. sakei* strains that were shown to be unable to grow on NANA (Anba-Mondoloni *et al.*, 2013). Other genes thought to play a role in adaptation to the meat niche include bacteriocin-associated genes, which allow these strains to compete on the meat surface, and transporters involved in iron or haem uptake (Chaillou *et al.*, 2009). Red meat is particularly rich in iron and *L. sakei* has been shown to transport haem or haem-carrier molecules (Zagorec and Champomier-Vergès, 2017).

The sourdough environment

Lactobacillus species are commonly associated with natural sourdough fermentation and are also used as biopreservative agents for extending the quality and shelf-life of sourdough breads. More than 60 different species of lactobacilli have been identified from sourdoughs (Gobbetti *et al.*, 2016), including *Lactobacillus sanfransicences*, *Lactobacillus reuteri*, *Lactobacillus brevis* and *Lactobacillus zymae* among others. *L. sanfransicences* is solely associated with the man-made environment of traditional sourdough fermentations. The species has not been isolated from other environmental niches and is therefore, quite specialized in its adaptation (Vogel *et al.*, 2011). Genome sequence analysis of *L. sanfranciscensis* TMW 1.1304 isolated from an industrial sourdough fermentation revealed several features which suggest adaptation to the sourdough environment (Vogel *et al.*, 2011). The organism contained one of the smallest genomes within the *Lactobacillus genus*, at 1.3 Mb, and the highest density of rRNA operons per Mb genome among all known bacteria capable of autonomous growth (Vogel *et al.*, 2011). It has been suggested that these characteristics are important for the rapid growth rate of the species in sourdough and the ability to respond to favourable growth conditions in the environment. In terms of metabolic capacity, the sequenced strain had the genetic potential to synthesize *de novo* several amino acids which are scarce in the wheat-based habitat, including aspartate, asparagine and glutamate. The proteolytic system of this species comprises a large number of peptidases, proteases and transport systems but lacks an extracellular protease as would be found in many dairy lactobacilli, reflecting the high dependency of *L. sanfranciscensis* on the protein-rich environment of sourdough.

In terms of niche adaptation, there is significant overlap between strains from sourdough and human intestine ecosystems. *L. reuteri* is an interesting case as this species, while present in sourdough, is also a dominant member of the intestinal microbiota of vertebrates (Duar *et al.*, 2017a). The species is phylogenetically differentiated into host-adapted lineages comprising rodent isolates (lineage I and III), human isolates (lineage II and IV), pig isolates (lineage IV and V) and poultry isolates (lineage VI). Sourdough isolates cluster with one or other of these host-adapted lineages, suggesting that adaptation to the sourdough environment is a recent evolutionary event. In terms of gene content, however, sourdough isolates cluster separately. A 2015 comparative genomics study of 16 strains of *L. reuteri* attempted to understand how the intestinal strains adapted to the sourdough environment and to identify genes which were unique to the sourdough isolates (Zheng *et al.*, 2015). Overall, it was demonstrated that gene loss and horizontal gene transfer played a major role in the adaptation of the species to the sourdough environment. The enrichment for genes coding for energy conversion and carbohydrate metabolism in the genomes of

sourdough isolates indicated positive selection in this environment. These genes included maltose phosphorylase, alcohol dehydrogenase and lactate dehydrogenase, genes known to contribute to competitiveness in the cereal environment. Genes common to all sourdough isolates but absent from other strains included those responsible for reutericyclin production. Reutericyclin is an antimicrobial compound that is structurally but not functionally related to naturally occurring tetramic acids and it was proposed that it may contribute to the competitiveness of the organism in sourdough. However, this has not been demonstrated in studies where a reutericyclin-negative derivative of the sourdough isolate TMW 1.656 was found to be as competitive as its wild-type parental strain (Lin *et al.*, 2015).

The plant niche

The microbes that are found living on plants are known as endophytes. These organisms generally live in a symbiotic relationship with the plant, where the microbes contribute to plant fitness and development and the plant provides the microbes with a reliable and constant source of nutrients and protection from stress (Mercado-Blanco, 2015). Plant matter is associated with native LAB including *Lactobacillus* species, few of which are known to be endophytic, while most are contaminants or are deliberately introduced, in particular for the preservation of vegetables for human consumption, and grass and maize for animal feed. Whichever the route to their presence in this environment, plant-associated species display particular traits that contribute to improving the quality, shelf-life, nutritional, and sensory attributes of plant-based food products for human and animal consumption.

Preservation of plant-based substrates through fermentation includes the process of ensiling, an effective method for maintaining the integrity and quality of forage crops used as livestock feed. During the process, epiphytic microbes ferment the plant material by acidification under anaerobic conditions, inhibiting the growth of undesirable species. *Lactobacillus buchneri* is a common member of the silage microbiome. Genome sequence analysis of this heterofermentative species has shown the absence of key enzymes necessary for glycolysis but the presence of a complete phosphoketolase pathway, thus confirming the species as an obligate heterofermenter (Heinl and Grabherr, 2017). As such, the organism, along with other lactobacilli, has been applied as a supplement during silage fermentation to improve the fermentation process and the stability of the silage. Heterofermentative species are more suitable than homofermentative species for this purpose as the additional by-products of fermentation provide additional protection against undesirable microbes, e.g. the anti-fungal properties of acetic acid. Niche adaptation in *L. buchneri* can also be demonstrated by its resistance to stresses encountered in the silage environment such as ethanol and oxygen. Transcriptomic studies revealed the up-regulation of oxidases, peroxidases and chaperone proteins in response to stresses of this nature. The presence of an S-layer is also thought to contribute to the physical robustness of the organism in this challenging environment (Heinl and Grabherr, 2017). Other species associated with silage fermentation include *Lactobacillus plantarum*, *L. brevis* and *Lactobacillus hilgardii*. A recently discovered species, *Lactobacillus hokkaidonensis*, a member of the *Lactobacillus vaccinostercus* group isolated from silage fermentation in a subarctic region of Japan (Tanizawa *et al.*, 2015), demonstrates remarkable psychrotolerance. In cold weather conditions, the impaired ability of LAB to produce sufficient acid to lower the pH can result in lower quality silage. While protection from low temperatures involves a wide range of biological systems which are difficult to identify through genomics alone, several factors were identified on the genome of *L. hokkaidonensis*, including the potential for accumulation of compatible solutes and the synthesis of glutathione, which could contribute to its metabolic activity at cold temperatures. Four transporters most likely responsible for the uptake of osmolytes were identified, similar to transporters of *L. sakei*, an organism that is also considered psychrotrophic. A bifunctional glutathione synthase encoded in the integrated and conjugative elements (ICE) region was also discovered. The presence of this gene system in *L. hokkaidonensis* and other psychrotolerant species such as *L. coryniformis* suggests that glutathione may facilitate psychrotolerance in both species (Tanizawa *et al.*, 2015).

Lactobacillus plantarum is a versatile species found in a variety of ecological niches, ranging from plants, to the gastro-intestinal tracts of human and

animals, as well as food materials, such as meat, fish, vegetables and raw or fermented dairy products. Its genome size of approximately 3.3 Mb is among the largest known for an LAB (Makarova *et al.*, 2006; Makarova and Koonin, 2007) and as previously mentioned, it is proposed that a larger genome is related to the diversity of environmental niches in which *L. plantarum* is encountered. Nonetheless, this bacterium is most frequently found in the fermentation of plant-derived raw material. It is the species most often found associated with the fermentation of plant material such as cocoa, olives, grape musts and sorghum, due to its ability to degrade tannins (Jiménez *et al.*, 2013). The species possesses the enzyme tannase (tannin acyl hydrolase, EC 3.1.1.20), which catalyses the hydrolysis of ester linkages in tannin to produce gallic acid and glucose. The gallic acid is then further decarboxylated to the less-toxic pyrogallol. Genes encoding an intracellular tannase and a gallate decarboxylase have been identified on the chromosome of *L. plantarum* WCFS1. While these genes are physically separated on the genome and transcribed independently, they are subject to common regulation (Jiménez *et al.*, 2013, 2014). A study on the adaptation of *L. plantarum* C2 to the plant-like conditions of carrot juice and pineapple juice demonstrated that the strain senses the plant stimulus and adjusts its carbohydrate metabolism to fit, towards pathways involving the metabolism and catabolism of amino acids (Filannino *et al.*, 2016). This could increase the strain's capacity to compete in diverse environmental conditions. Comparative genomic analysis of 54 strains of *L. plantarum* isolated from a variety of sources attempted to understand the link between evolution and the ecological versatility of this organism (Martino *et al.*, 2016). The study revealed an absence of specific signatures in the genome associated with a particular ecological niche, reflecting the nomadic lifestyle of this particular species. This genomic flexibility allows the organism to effectively grow in a wide range of habitats, highlighting the generalist nature of *L. plantarum* (Martino *et al.*, 2016).

The human gastrointestinal tract as an environmental niche

Lactobacilli are essential members of a healthy human microbiota and are found at various niches in the human body (Fig. 1.3). Undoubtedly, the adaptation of lactobacilli to the human gut is the most analysed of all environmental niches, with many studies performed on probiotic strains, which are mostly used for treatment of gut disorders. Although recently it was proposed that colonization of neonates occurs *in utero* (Collado *et al.*, 2016), currently it is still widely accepted that the colonization of the GIT starts after birth and its development depends on the infant's diet, hygiene level and other factors (Azcarate-Peril *et al.*, 2008). The human GIT presents a challenging environment and strains successfully colonizing this niche require the ability to adhere to the mucosal surfaces of the gut, to compete with other bacteria for the available nutrients and to adapt to the presence of host-derived molecules such as bile salts (Duar *et al.*, 2017b). In terms of their original habitat, strains present in the GIT can roughly be divided in two groups. The majority of GIT isolates detectable in faeces, especially of humans, comprise transient, allochthonous microbiota originating from different sources, mostly food or saliva. In contrast, a smaller group of GIT isolates comprise actual gut inhabitants, often designated as autochthonous species and rarely isolated from other sources not connected with the GIT (Reuter, 2001). For example, 17 *Lactobacillus* species are putative inhabitants of the human gut, but only a limited number (*Lactobacillus gasseri*, *Lactobacillus salivarius*, *Lactobacillus ruminis* and *L. reuteri*) are actual inhabitants of the GIT, while most others are transient microorganisms originating from fermented food products (Walter, 2008).

The oral cavity is part of the GIT in a broader sense, and the mouth represents the entry port of food to the lower parts of the digestive tract. Numerous bacterial species including lactobacilli colonize the teeth, gums, saliva and tongue (Douillard and de Vos, 2014). At the birth of vaginally derived neonates, the sterile mouth of the baby is colonized by lactobacilli present in mother's vagina. However, these lactobacilli are transient and not sustained in the baby's mouth after one month. Lactobacilli are also found in the mouths of breastfed infants, but after weaning and prior to tooth emergence, lactobacilli are rarely found in the oral cavity of infants. Later in life, the most obvious sources of lactobacilli in the oral niche are food or other infected humans (Caufield, *et al.*, 2015).

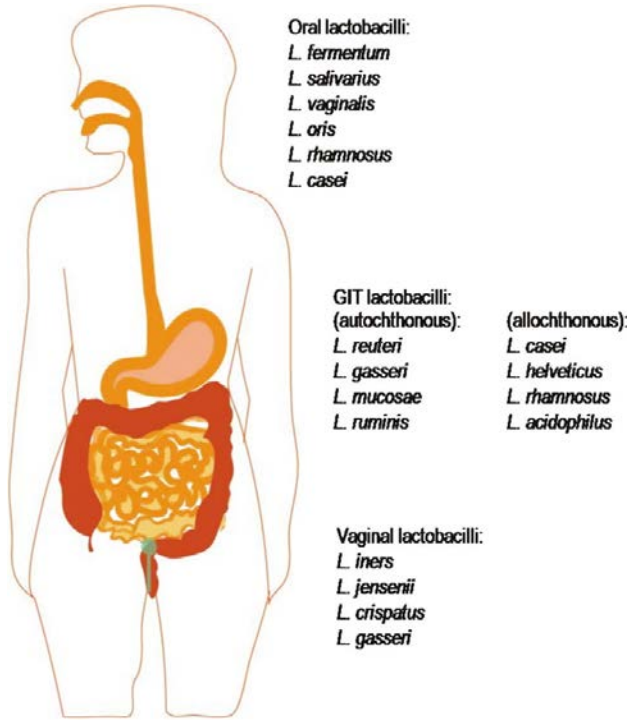

Figure 1.3 Locations of *Lactobacillus* species in various niches of the human body.

Upon analysis of the oral microbiota of older children and adults, two major populations of oral lactobacilli are generally distinguishable. The first of them consists of transient lactobacilli connected with food intake, and these are often a source of lactobacilli isolated from faeces (Dal Bello and Hertel, 2006). However, a key ecological determinant for the sustained colonization of lactobacilli in the oral cavity is the presence of dental caries (Caufield, *et al.*, 2015). Species usually connected with caries lesions belong to *L. gasseri*, *L. rhamnosus*, *Lactobacillus vaginalis*, *Lactobacillus crispatus*, *L. fermentum*, *Lactobacillus salivarius*, *Lactobacillus oris* and *L. casei* (Caufield, *et al.*, 2015, Yang, *et al.*, 2010). The phylogenetic relationships between these species show that lactobacilli associated with dental caries belong to different phylogenetic groups, and adaptation to the caries niche appears to be independent in different lineages (Caufield, *et al.*, 2015). Dental caries are caused by acidogenic bacteria that produce lactic acid as a result of anaerobic fermentation of carbohydrates. However, lactobacilli are not detected as a major cause of initial caries lesions development, but rather considered as secondary invaders of lesions already formed by *Streptococcus mutans*. The initial colonization of the teeth surface by *S. mutans* creates the necessary niche capable of mechanistically retaining both lactobacilli ('retentative' niche) and food, a source of carbohydrates. In light of caries developments, several characteristics enable survival and persistence of oral lactobacilli in caries lesions. Apart from the obvious tolerance to low pH, some isolates connected with dental caries possess collagen-binding proteins, which could help them to endure in caries lesions. Another feature contributing to caries adaptation of lactobacilli is their relative tolerance to fluorides, and the ability to metabolize xylitol, mannitol and sorbitol, often used as sweeteners and anticaries agents, thus actually promoting caries progression in lesions dominated by lactobacilli (Caufield, *et al.*, 2015). When genomes of *L. rhamnosus* strains isolated from dental pulp were analysed, it was shown they possessed up to 250 unique genes, coding for transcriptional regulators, ferric ion ABC transporters and exopolysaccharides (EPS), but no genes encoding pili components were detected. This suggested that EPS could be important for biofilm formation and promotion of caries, while pili seem not to be essential for the persistence in

the oral niche (Douillard and de Vos, 2014). From the available data, it could be concluded that caries lesions can promote the development of lactobacilli populations with specific phenotypes supporting their persistence in the oral cavity and constantly supplying lower parts of GIT with lactobacilli. However, oral niche adaptability has been the focus of a small number of studies and further analysis of the specific genomic content of common caries-related lactobacilli is necessary to describe specific mechanisms of accommodation to the oral niche.

Some of the common features of gut isolates were identified through a series of genomic comparison studies of *Lactobacillus* species isolated from various niches. These studies aimed to distinguish between isolates of different origin and are beneficial in pointing out some features that are important for gut survival. They do not, however, provide evidence of typical niche specialization, since as previously mentioned, the GIT represents a transient environmental niche rather than the true niche for these species. In species that are considered as free-living or nomadic (*L. plantarum*, *L. casei* group), or frequently used in dairy processing and as probiotics (*L. helveticus*, *L. acidophilus*), several features were distinguished among gut isolates or strains used as probiotics. A genome-scale study of *L. helveticus* strains MTCC 5463 (a probiotic strain) and DPC4571 (a dairy isolate) showed that the MTCC 5463 genome possessed multiple genes for bile salt hydrolase (bsh), important for survival in the bile-rich environment of the GIT. In order to survive in conditions of constant competition for nutrients, the probiotic strain carries a higher number of starvation-induced genes compared to the dairy isolate (Senan *et al.*, 2014). Another study performed on *L. helveticus* strains confirmed that, in probiotic strain R0052, mucus-binding proteins were present, since they would be essential for survival and residence of the strain in the gut (Cremonesi *et al.*, 2012). In the previously mentioned study that analysed 100 *L. rhamnosus* strains, intestinal and probiotic strains shared similarities with other human isolates. These strains were bile resistant, pili-possessing and L-fucose-utilizing, all characteristics important for intestinal tract survival (Douillard *et al.*, 2013). In the study of 11 fully sequenced LAB (10 of which were lactobacilli) originating from different sources (three dairy, five gut and three multiniche LAB), nine genes grouped in four classes (sugar metabolism, the proteolytic system, restriction–modification systems and bile salt hydrolysis) were identified as niche-determinative as they insured survival in the gut or dairy environments (O'Sullivan *et al.*, 2009). All of these studies pointed to characteristics that enable survival of lactobacilli associated with the GIT niche: a wide spectrum of genes encoding transporters to make the best out of available nutrients; genes encoding for acid and bile resistance and finally genes conferring interaction and signalling with the host such as pili containing mucus-binding proteins, but these are only providing evidence of strain (rather than species) adaptation. Pili and mucus-binding proteins and other mediators of bacterial adhesion to the gut mucosa have been confirmed in strains isolated from the GIT, and are often seen as one of the prerequisites for the persistence of probiotic strains. The stratified squamous epithelia is not present in the human stomach, and the colonization of mucosal surfaces by GIT microbiota is very limited in humans since significant epithelial associations of gut bacteria or biofilms have not been described. Instead, commensal bacteria appear to live in suspension with limited contact with epithelial cells. Rapid generation times are therefore vital for the bacteria to avoid washout. How lactobacilli facilitate rapid growth in the human intestinal tract remains unclear. This is why the majority of the traditional probiotic strains (*L. acidophilus*, *L. casei* group, *L. delbrueckii*, *L. brevis*, *L. plantarum*, *L. johnsonii*, *L. fermentum*) are probably allochthonous to the human intestinal tract and although they are present in faecal samples, they are not able to form stable populations in the human gut. However, since probiotic effects do not necessarily depend on the persistence, their probiotic function remains unimpaired if constant supply of probiotics is maintained (Walter, 2008). Interestingly, even when autochthonous strains are used as probiotics, their persistence is not significantly better than other allochthonous probiotics. When the persistence of the probiotic species *L. reuteri*, *L. mucosae* (both of which are recognized as autochthonous) and *L. acidophilus* (allochthonous) were analysed, the cell numbers of two autochthonous strains were higher than for the allochthonous strain, suggesting that autochthonous probiotics were able to reach a higher 'effective' dose in the gut. This is important in cases where the probiotic

effect depends on the number of present bacteria. The restricted ability of *L. acidophilus* to form stable populations in the human gut is likely due to the absence of specific adaptive features, which are present in *L. reuteri* and *L. mucosae*. Nevertheless, autochthonous strains were not more persistent over time. This implies that even if a probiotic strains belongs to the autochthonous microbiota of an individual, it might be impossible to establish a stable population in the gut (Frese *et al.*, 2012).

One of the human autochthonous species is *L. gasseri*, and the molecular basis for its autochthony has been reported in analysis of the genome sequence of human isolate *L. gasseri* ATCC33323. With regard to sugar metabolism, its diverse range of PTS transporters and sugar hydrolases for mono- and di-saccharides imply its adaptation to the upper GIT, where these sugars are present. However, complex sugars are not metabolised by this strain. In addition, two *bsh* genes detected increase the competitiveness of the strains and support its survival in the gut. Up to 14 putative mucus-binding proteins were detected, facilitating the adhesion of the strain, but not all of them possessed a signal peptide, meaning they might be either non-functional or secreted by other mechanism. In addition, an EPS cluster of 16 genes was detected, and EPS polymers have been shown to contribute to successful adherence. Additional gut-specialization features included the ability to degrade oxalate, commonly present in dietary sources and also produced by intestinal microbiota from precursors. Besides that, a broad range of molecular chaperones and chaperonins that protect proteins from irreversible aggregation during stress conditions were detected. Interestingly, although bacteriocins are helpful in maintaining stable populations and in bacterial competition, *L. gasseri* does not encode for bacteriocin peptides (Azcarate-Peril *et al.*, 2008). Similarly, a lack of bacteriocin production or transport was observed in another human autochthonous strain, *L. ruminis* ATCC 25644 (Forde *et al.*, 2011), suggesting that this trait is not an essential feature of genuine gut lactobacilli.

Another species considered to be autochthonous for the human (and animal) GIT is *L. reuteri* (Reuter, 2001) but ecological strategies of this species are different in humans and animals. For example, while in animals *L. reuteri* forms large populations in the proximal regions of GIT, in humans they use mainly mucus-binding adhesion, which results in relatively low prevalence in the human population (Wegmann *et al.*, 2015). According to multi-locus sequence analysis (MLSA) of *L. reuteri* from different hosts (Oh *et al.*, 2010), human isolates of *L. reuteri* belong to two distinct clades. Group II is solely comprised of human isolates, while group VI includes both human and poultry isolates. In group II, one of the most important observations was the absence of many genes encoding for biofilm formation and adhesion, which could mean that in humans, *L. reuteri* develops a 'planktonic' lifestyle in more distal regions of the human gut and limited interactions with gut epithelium. Since no large surface proteins were detected, group II isolates probably persists in the gut by fast multiplication. Interestingly, the *pdu–cbi–cob–hem* cluster was conserved within the human strains. This cluster encodes for cobalamin synthesis, 1,2-propanediol fermentation and reuterin production. Because nutrients are scarcely available in the colon, the ability of *L. reuteri* to use 1,2-propanediol might be an important factor of survival in the colon. The production of reuterin might contribute to the fitness of *L. reuteri* in the human gut through inhibition of competitors. Strains belonging to human II group have highly similar genetic content, are highly conserved and are clonally related. In addition, they show signs of reductive evolution, since pseudogene formation and gene deletions have been detected (Frese *et al.*, 2011). In a subsequent study, that aimed to confirm host specialization, the administration of human II isolates facilitated the presence of the isolates in human faecal samples, while after administration of human VI isolates, they were undetectable in human faeces. The genomic analysis of two subgroups of group VI (chicken and human) showed that while poultry VI strains possessed an open genome with a large number of strain-specific genes, human VI isolates possessed very few unique genes and showed essentially a closed pangenome and showed very little genomic variation, similarly to group II. It was proposed that strains from lineage VI can become transiently associated with humans. These findings suggest that a single clone acquired specific traits that allowed a temporary migration to humans, such as genes encoding antibiotic resistance and capsular polysaccharide synthesis (Duar *et al.*, 2017a).

The animal gastrointestinal tract as an environmental niche

The vertebrate GIT niche

Although most often studied in relation to the GIT of humans, lactobacilli are also regularly isolated from the digestive tract of a variety of insects and vertebrates, such as birds, rodents, and farm animals, but the host range is probably broader (Duar, *et al.*, 2017a). Unlike in humans, where limited gut niche specialization has been observed, most lactobacilli present in the gut of mice, rats, pigs and chickens are autochthonous, since they form stable populations throughout the life of the host. The stomach of pigs, mice and rats and the crops of birds are lined, at least partially, with squamous stratified epithelium and form a layer of bacterial cells, often embedded in a matrix of extracellular polymeric substances. The features of the autochthonous species that enable specialization to animal hosts include adherence due to large surface proteins and EPS production that contributes to cell aggregation. In addition, some features, such as IgA protease are important for survival of innate or adaptive immune response of the host (Walter, 2008).

In the above mentioned study (Oh *et al.*, 2010), the phylogeny of *L. reuteri* strains isolated from six different host species (human, mouse, rat, pig, chicken and turkey) was assessed by MLSA. A significant level of heterogeneity that corresponded to the host origin was observed. Apart from two previously mentioned groups (group II of human isolates, and group VI of human and poultry isolates), groups I and III included rodent isolates, groups IV and V included pig isolates. In addition, this grouping was later confirmed in genomic analysis (Duar *et al.*, 2017a). This result suggested that different conditions present within GIT of different hosts facilitated diversification of strains in host specific lineages and the development of a stable relationship of *L. reuteri* with vertebrate hosts during a long period of close associations (Frese *et al.*, 2011). For example, the urease cluster was highly conserved among rodent isolates, and urease is important for survival in acidic conditions in forestomach. In addition, 11 large surface proteins were detected almost exclusively in rodent strains. Most of them are potentially involved in epithelial adhesions and biofilm formations. The xylose operon was highly conserved in rodents and porcine strains. Xylose could be an important substrate for gut bacteria as a plant-derived sugar present in straw and bran. The SecA2 cluster was detected in most strains from rodents and pigs, while it is rare in human and poultry isolates. Since mobile elements were present within the cluster, it is assumed that it was acquired in HGT event. This secretion system could be involved in secretion of several adjacent proteins, one of which is serine rich, and probably possesses adhesion function. An important note is that rodent-specific genes were not conserved among all rodent strains, and adaptation to murine GIT is defined by a rodent-specific accessory genome, reflecting a plasticity of rodent *L. reuteri* genomes and higher diversity among the host population, in contrast to previously described very limited variations among human isolates (Frese *et al.*, 2011).

A pangenome study of *L. reuteri* based on five genomes of pig GIT isolates (Wegmann *et al.*, 2015) confirmed that two distinct clades are observed (IV and V), as previously suggested (Oh *et al.*, 2010). No genes conserved in all pig isolates were found but genes specific for strains in one group were detected. They included genes encoding cell wall -associated proteins, EPS biosynthetic enzymes, phage-related functions, mobile elements and DNA metabolic enzymes. This means that the two populations of pig-associated *L. reuteri* evolved separately, through a process driven by differences in host genotypes or dietary components (Wegmann *et al.*, 2015). In another study of *L. reuteri* strains, the total pangenome of 42 strains of different isolation source (26 of them of various origin previously sequenced and 16 pig isolates from the current study) was assessed in order to find specific genomic characteristics associated with porcine adaptation. All porcine isolates belonged to group IV according to the MLSA clustering. The phylogenetic clustering based on RAST subsystem comparison showed that all porcine strains grouped together, while strains of other origins were dispersed and not categorized for each host. Six genes (three encoding hypothetical and three encoding cell surface proteins) were identified only in the porcine group, suggesting that surface proteins contribute to host adaptation. Also, these host-specific genes were probably obtained by horizontal gene transfer, since transposase genes

were co-localized. Compared with strains originating from other niches, pig isolates were missing six genes, four of which were associated with several pathways for energy production, but they were not found to be essential for growth and survival. Interestingly, differentiation between pig isolates with high (HS) and low (LS) anti-pathogenic effect (determined based on the activity against two frequent swine pathogens) showed that the HS group had the full cluster for production of reuterin, while in some strains in the LS group, a subsystem for citrate metabolism transport and regulation was determined. Both of these traits could represent specific adaptation leading to survival in the pig as a host (Lee *et al*., 2017a).

Another species of *Lactobacillus* that is commonly isolated from the GIT of vertebrates is *L. salivarius.* In a comparative study that analysed 35 *L. salivarius* strains from pigs, humans and chicken, strains clustered based on the host from they were isolated. Two main strategies to adapt to a specific host were increasing adhesion and colonization abilities and producing more energy through changes in carbohydrate utilization. Several molecules contributed to this diversification of isolates. In each of the groups of isolates, specific genes for cell surface proteins that facilitate adhesion to a host habitat were detected. These include mucus-binding proteins, or less precisely annotated molecules, such as cell surface proteins or hypothetical secreted protein, for which adhesion function has been assigned according to functional domains detected in proteins. Additionally, extracellular components are considered responsible for niche adaptation through interactions with hosts. The clustering based on orthologues encoding extracellular proteins showed that bacteria grouped by the hosts from which they were isolated. Similarly, the divergence of strains associated with the host was observed based on the comparison of 24 conserved EPS genes. Additionally, differences in a host's dietary habits facilitated differences in energy consumption in specific lineages. For instance, in pig isolates, genes involved in carbohydrate metabolism via the citric-acid cycle were detected, indicating that these genes contribute to the energy production for the growth of pig isolates. In the genomes of chicken isolates, genes related to acetate and lactate metabolism were specific. Interestingly, *L. salivarius* strains isolated from humans have no host specific genes for adhesion to the habitat and for energy production. Because humans intake various foods, genes required only in certain circumstances are not essential. In contrast, the diet of pigs and chickens is based on uniform formulated feed, so the genomic content for energy metabolism of commensal bacteria is much less variable. In terms of bile salt hydrolases, while human and chicken isolates possess one or two *bsh* genes, most of pig isolates have three genes (Lee *et al*., 2017b).

The invertebrate GIT niche

In its prominent role as a pollinator, the honey bee is the key species for agricultural production. The recent decline of populations of honey bees and bumble bees triggered the need for a better understanding of the bees' microbiota and its potential to benefit the host health (Engel *et al*., 2012). The honey-bee gut microbiota consists of eight distinct phylotypes, five Gram negative (which will not be discussed here), and three Gram positive, two of which refer to lactobacilli, designated as Firm 4 and Firm 5 (Firmicutes) and one referring to bifidobacteria (Engel *et al*., 2012). Lactobacilli in the Firm 4 group include *L. mellifer* and *L. mellis*, while Firm 5 includes *L. helsinborgensis*, *L. melliventris*, *L. kimbladii*, *L. kullabergenis* and *L. apis* (Ellegaard *et al*., 2015; Moran, 2015). In the gut of the honey bee, lactobacilli are isolated from the crop (*L. kunkeei*, and both Firm 4 and Firm 5 groups) (Ellegaard *et al*., 2015), but they mostly dominate in distal parts of the insect's gut (hindgut) (Kwong and Moran, 2016). The genomes of lactobacilli commonly associated with honey bees are characterized by small sizes (1.54 Mbp in *L. apis* to 2.13 Mbp in *L. kimbladii* (Ellegaard *et al*., 2015)). The genomic analysis of lactobacilli and bifidobacteria revealed several features important for adaptation to this host. Interestingly, in all three groups, a significant level of synteny was observed. Each group was characterized by novel outer surface protein families probably involved in the interactions with the host or other bacteria.

With regard to differences among the two groups of lactobacilli, one of the gene groups that was present in Firm 4 (and Bifido) group, but not in Firm 5, was *cydABCD*, which is involved in aerobic respiration and thus is important for colonization of the gut and possibly reflects the adaptation to different microhabitats. Firm 4 group also possessed

11 conserved group-specific protein families, which are, based on their domains, involved in co-aggregation with other bacteria. Additionally, some of these domains have been previously identified in *L. plantarum* and were connected with utilization of complex plant polysaccharides. However, the most important feature contributing to niche specialisation of honey-bee lactobacilli refers to their sugar metabolism capacities. In Firm 5 group, the accessory genes were predominantly PTS transporters, and large majority of them coded for mannose–sorbose–fructose family transporters. Some of the species (*L. kullabergensis* and *L. kimbladii*) possessed up to 70 genes for this transporter family. Interestingly, PTS transporters were often found in genomic islands (GI) with a lack of sequence similarity between the genomes, and were co-localized with other sugar-metabolism-related genes (glucosidases, hydrolases, isomerases etc) suggesting that these GI code mainly for carbohydrate-related functions. Deeper analysis of the evolutionary relationships of the PTS genes showed that they have undergone an extreme expansion which preceded the diversification of Firm 5 strains and was followed by loss, recombination, diversification and transfer between groups 4 and 5 (Ellegaard *et al.*, 2015). Similar findings were observed in the metagenomic study of gut microbiota of honey bee, which found that cluster of orthologous genes (COG) for carbohydrate metabolism and transport was significantly enriched, referring to the adaptation to the diet and gut environment. The large number of different PTS were present in Firm groups, suggesting that they metabolize a variety of sugars, and mannose transporters are the most abundant and characterized by wide substrate specificity. Various nectar and pollen sugars cannot be metabolized or are toxic to the honey bees, and metabolism of these sugars by the gut microbiota could be critical for detoxification of food components. In regard to EPS production no *eps* clusters were identified within Firm 5 group, but Firm 4 contained genes for dTDP-rhamnose synthesis, and multiple glycosyl transferases, both of which are involved in EPS production (Engel *et al.*, 2012).

As previously said, *Lactobacillus kunkeei* is usually present in the crop of the honey bee, and also in the larval gut, nectar, honey and hive, however it is absent from adult hindgut (Moran, 2015). It is classified as a sole obligate fructophilic LAB (FLAB), meaning that it grows well on D-fructose, but poorly on D-glucose. Analysis of the 16 sequenced genomes of the species showed that the genome sizes were approximately 1.54 Mbp, significantly smaller compared to the majority of other lactobacilli and among the smallest genomes in bee isolates. In correlation with poor carbohydrate metabolic ability of the species, *L. kunkeei* genomes also had fewer genes for carbohydrate metabolism and transport compared to other small *Lactobacillus* genomes, lacking most of the genes used for TCA cycle and complete missing of PTS system. Additionally, over-representation of genes for catabolism and anabolism of fatty acids and amino acid biosynthesis was observed. An important observation was made in regard to ADH/ALDH protein, a bifunctional alcohol/acetaldehyde-dehydrogenase, one of the key enzymes for D-glucose metabolism and ethanol production in the phosphoketolase pathway of heterofermentative LAB. *Fructobacillus* spp. have been reported as the only heterofermentative LAB that lack *adhE* gene and ADH/ALDH activities, which is in connection with their poor glucose metabolism and low ethanol production. Although *L. kunkeei* has similar biochemical characteristics, shorter *adhE* genes encoding only ALDH domain, but not ADH domain were identified. This means that in glucose metabolism of *L. kunkeei*, the lack of ADH activity is followed by acetate production (Maeno *et al.*, 2016). More recently, another *Lactobacillus* (*L. apinorum*) has been included in the group of fructophilic LAB (Maeno *et al.*, 2017), contributing to observation of even higher level of specialization of some insect-related lactobacilli.

The vaginal niche

Lactobacilli represent a common member of the complex vaginal microbiota in women from different ethnic groups and living in different geographical locations (Douillard and de Vos, 2014). The main *Lactobacillus* species that constitute the vaginal flora are *L. crispatus*, *L. jensenii*, *L. gasseri* and *L. iners*, and interestingly, these species are rarely isolated from other habitats (Martín *et al.*, 2008; Mendes-Soares *et al.*, 2014). Unlike in the GIT where different lactobacilli cohabit due to the nutritional adaptations and resource partitioning (Tannock *et al.*, 2012), in the vaginal cavity one species usually dominates the community and

stable co-existence of vaginal lactobacilli is not often observed. The four most commonly found vaginal *Lactobacillus* species interact with the environment in different ways which enable them to establish a stable population of single species dominance (Mendes-Soares *et al.*, 2014). On the other hand, some authors argue that species can also share niche space temporarily through conditional differentiation, taking advantage of the ability to accommodate the range of environmental conditions, due to which the abundance of the species is determined by factors that influence their competitive interactions (France *et al.*, 2016).

The vaginal microbiome in healthy women consists of five cluster groups, four of which are dominated by lactobacilli, while the fifth cluster has low numbers of LAB and high numbers of strict anaerobes. The key ecological stamp in all of the identified clusters is the production of lactic acid (Ravel *et al.*, 2011). In addition, no single core vaginal microbiome was observed, but more precisely, core genomes were defined in the community groups. The core functions are conserved among these communities, and that functional redundancy would be associated with increased community reliability to adapt to the environmental changes (Ravel *et al.*, 2011).

Analysis of the genomic features of vaginal-related species showed that their genomes are significantly smaller and with lower GC content than species characteristic for other ecological niches, suggesting a high level of host adaptation and the development of a symbiotic lifestyle. Analysis of the protein families revealed that the species possess different mechanisms to interact with their environment. The number of proteins present exclusively in each of the vaginal species was the highest in *L. crispatus* and the lowest in *L. iners*, which also have the largest and the smallest genomes of vaginal lactobacilli, respectively. It was found, for example, that *L. iners* possesses the gene for thiol-activated cytolysin (inerolysin), which is identified as up-regulated during bacterial vaginosis (BV) (Mendes-Soares *et al.*, 2014). This raises the possibility that the *L. iners*-encoded CDS may play an unappreciated role in BV and might contribute to the pathogenesis of the condition (Macklaim *et al.*, 2013), although another study observed this trait as an adaptation to cases of scare nutrient availability in the vaginal niche (France *et al.*, 2016). Interestingly, all analysed vaginal *L. gasseri* strains encode for the bacteriocin pediocin (Mendes-Soares *et al.*, 2014). The *L. crispatus* strains were found to have a unique DNA polymerase, bacteriocin and toxin–antitoxin systems and genes encoding mobile genetic elements, especially transposases that contribute to its large genome size. Additionally, *L. crispatus* had 26 protein families that are not found in other vaginal lactobacilli, two of which possess cell wall anchoring domains (Mendes-Soares *et al.*, 2014). Additionally, lysogeny of phage particles was observed recently in most of the vaginal isolates of *L. crispatus*, which could explain the large number of genes encoding mobile genetic elements (Derrien and van Hylckama Vlieg, 2015).

In a comparative-genome study of 10 *L. crispatus* strains, several features important for successful colonization of the vaginal cavity were reported. In regard to vaginal health, the most interesting features include genes for EPS biosynthesis, which could be important for adhesion, biofilm formation and competitive exclusion of pathogens. Eight out of ten analysed strains produced EPS, but strains produced EPS with different sugar monomers and glycosidic linkages. In addition, *L. crispatus* were shown to possess genes encoding bacteriocins similar to enterolysin A, helveticin J and pediocin J, thus contributing to protective function against pathogens. The metabolic route for generation of hydrogen peroxide from pyruvate was ubiquitously present, further enhancing the protective role of *L. crispatus* against pathogens. The conserved pathways were annotated for the metabolism glucose and mannose. Although no complete routes for the metabolism of glycogen were identified, seven vaginal strains were discovered to carry a type I pullulanase gene, which could contribute to the degradation of glycogen. Although strains were able to synthesize seven amino acids *de novo*, overall the dependency on external supplies of amino acids was shown. In terms of adhesion to the surface of the vagina, 103 proteins were identified with the potential to enable colonization of the host. Approximately 30 putative S-layer protein-encoding genes that could potentially contribute to the bacterial adhesion were reported (Ojala *et al.*, 2014). Importantly, several molecular factors in *L. crispatus* were shown to be antagonistic to the vaginal pathogen *Gardenella vaginalis*, and prevent its adhesion, most important of which is *Lactobacillus*

epithelium adhesin (LEA), which is shown to be universally present in *L. crispatus* strains (Ojala *et al.*, 2014).

The comparison of genome characteristics of *L. crispatus* and *L. iners* revealed ecological factors that drive niche partitioning by these species. Both of the species rely on fermentation of carbohydrates as sources of energy. Genes encoding glucose, trehalose, maltose and mannose were identified in both species, while *L. crispatus* additionally can ferment lactose, galactose, fructose and sucrose. *L. crispatus* and *L. iners* have similar numbers of genes related to metabolism of some amino acids, but *L. crispatus* also has the genetic capability to transport and breakdown putrescine, a product of ornithine catabolism, suggesting that *L. iners* is more dependent on exogenous sources of amino acids. However, *L. iners* is able to produce inerlysin, a pore-forming cytolysin, which enables *L. iners* to liberate resources from the host cell, giving competitive advantage to this species in the vaginal environment when nutrients are scarce. The reported differences in the genetics of metabolism could be the determining factor competitive interactions between these two species (France *et al.*, 2016).

Concluding remarks

The extraordinary genomic diversity that defines the *Lactobacillus* genus has resulted from interactions with different environments and different genomes within those environments as these organisms have evolved to adapt to the various niches in which they are found. While no specific signature sequences have been identified which associate a particular species with a particular niche (Sun *et al.*, 2015), there is overwhelming evidence that remodelling of genomes through gene loss and acquisition has played a major role in the adaptation of this genus to nutritionally rich environments. Phylogenomic analysis has revealed that lifestyle categories, whether free-living, host adapted or 'nomadic', correlate with phylogenetic groupings (Duar *et al.*, 2017b). Many of the present-day species of *Lactobacillus* exhibit variation in their reliance on specific niches, having evolved from free-living ancestors. In general, the metabolic capabilities of species reflect their lifestyle adaptations. These capabilities have the potential to be exploited in a wide range of applications, as our growing knowledge on the capacities of the *Lactobacillus* genus reveal a largely, as yet, untapped resource. Exploitation of the diversity of lactobacilli could have significant implications for new product development. There are exciting new opportunities in the field of biotransformation, and additional opportunities to improve the application of this important genus in industrial and therapeutic applications.

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