Lifestyles in transition: Evolution and natural history of the genus *Lactobacillus*

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ABSTRACT

Lactobacillus species are found in nutrient-rich habitats associated with food, feed, plants, animals and humans. Due to their economic importance, their metabolism, genetics, and phylogeny have been extensively studied. However, past research primarily examined lactobacilli in experimental settings abstracted from any natural history and the ecological context in which these bacteria exist and evolve has received less attention. In this review, we synthesize phylogenetic, genomic and metabolic metadata of the *Lactobacillus* genus with findings from fine-scale phylogenetic and functional analyses of representative species to elucidate the evolution and natural history of its members. The available evidence indicates a high level of niche conservatism within the well-supported phylogenetic groups within the genus, with lifestyles ranging from free-living to strictly symbiotic. The findings are consistent with a model in which host-adapted *Lactobacillus* lineages evolved from free-living ancestors, with present-day species displaying substantial variations in terms of the reliance on environmental niches and the degree of host-specificity. This model can provide a framework for the elucidation of the natural and evolutionary history of *Lactobacillus* species and valuable information to improve the use of this important genus in industrial and therapeutic applications.

INTRODUCTION

Lactobacilli are fastidious gram-positive bacteria that populate nutrient-rich habitats associated with food, feed, plants, vertebrate and invertebrate animals, and humans. Owed to their use in food, in biotechnology and in therapeutic applications, lactobacilli have substantial economic importance. Consequently, research has focused on their role in food fermentations and spoilage (Chaillou *et al.* 2005; Gänzle and Ripari 2016; Stefanovic, Fitzgerald and McAuliffe 2017), biotechnological applications (Sun *et al.* 2015) and their functionality as ‘probiotics’, which are “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Marco, Pavan and Kleerebezem 2006; Lebeer, Vanderleyden and De Keersmaecker 2008; Bron, van Baarlen and Kleerebezem 2011; Hill *et al.* 2014).These studies have provided important information regarding the metabolism and functionality of a wide array of *Lactobacillus* species in food environments, in the gastrointestinal tract as well as their role in human and animal health. From an ecological and evolutionary perspective, however, these studies provide little insight, as they are conducted in experimental settings that are abstracted from any natural history. Food habitats are man-made and date back less than 14,000 years (Steinkraus 2002; Hayden, Canuel and Shanse 2013) which is short when considering that the associations of lactobacilli with plants and animals dates back millions of years (Tailliez 2001; Battistuzzi *et al.* 2004). Furthermore, most probiotic research has been conducted with *Lactobacillus* strains ‘allochthonous’ to the respective hosts in which they were studied (Walter 2008). We therefore lack information regarding the evolution of lifestyles in lactobacilli as it occurred in their true ecosystems in nature.

The genus *Lactobacillus* comprises more than 200 species characterized by a phylogenetic and metabolic diversity that exceeds that of a typical bacterial family (Sun *et al.* 2015). Recent phylogenetic analyses based on robust core genome phylogeny have revealed that lactobacilli can be subdivided into at least 24 phylogenetic groups (Zheng *et al.* 2015a); species of the genus *Pediococcus* form an integral part of the genus *Lactobacillus. A*ccordingly, lactobacilli have been referred to as the *Lactobacillus sensu lato* including pediococci, or the *Lactobacillus* Genus Complex to additionally include the related genera *Weissella, Leuconostoc, Oenococcus* and *Fructobacillus* (Sun *et al.* 2015; Zheng *et al.* 2015a) (BOX 1). The availability of genome sequences of lactobacilli has created a robust framework for large scale phylogenomic and comparative genomic analyses that can elucidate their evolution (Sun *et al.* 2015; Zheng *et al.* 2015a). In addition, population genomics and genetic analyses have allowed a detailed reconstruction of the evolutionary patterns in specific *Lactobacillus* species (Oh *et al.* 2010; Frese *et al.* 2011; McFrederick *et al.* 2013; Martino *et al.* 2016). If informed by an understanding of the metabolic traits of *Lactobacillus* groups and lineages, these analyses provide an opportunity to explore the ecological and evolutionary contexts in which these bacteria exist in nature and how their lifestyles have evolved. In this review, we compile the available genomic and metabolic metadata for the genus *Lactobacillus* to infer its evolution and natural history. Specifically, we apply a phylogenomic approach to infer the natural habitat and relate this analysis to metabolic, functional and fine-scale phylogenetic analyses of model species. We summarize the available information and discuss the lessons to be learned from an evolutionary and ecological understanding of lactobacilli for biotechnological and therapeutic applications.

BOX 1: Taxonomy of lactobacilli. Numerous classification schemes have been developed since the first description of the genus in 1901 (Beijerink 1901). Pioneering work by Orla-Jensen in 1919 introduced the earliest systematic categorization of the genus on the basis of its optimum growth temperature and the pathways for carbohydrate fermentation (Orla-Jensen 1919). Later revisions subdivided the genus based on fermentation characteristics into obligate homofermentative, facultative heterofermentative and obligate heterofermentative species (Hammes and Vogel 1995). This phenotype-based nomenclature, however, does not accommodate the pathway for pentose conversion to lactate as sole end product, does not reflect the grouping of lactobacilli with respect to their metabolic repertoire, and is inconsistent with the phylogenetic structure of the genus (Hammes and Vogel 1995; Pot *et al.* 2014, Gänzle, 2015). The phenotype based nomenclature was replaced by differentiation between homofermentative and heterofermentative species, which matches the metabolic and genetic repertoire as well as the phylogenetic relatedness of species (Gänzle 2015; Zheng *et al.* 2015a). After the development of molecular tools, many species have been renamed and some have been reclassified to other genera (Sharpe, Fryer and Smith 1966; Klein *et al.* 1998; Salvetti, Torriani and Felis 2012). The broader cluster of genera can be divided into 24 phylogenetic groups including *Pediococcus* species as integral members of lactobacilli. These groups share major metabolic traits (Pot *et al.* 2014; Zheng *et al.* 2015a) and are phylogenetically robust as they have been consistently established by 16S rRNA and core genome phylogeny (Hammes and Hertel 2006; Salvetti, Torriani and Felis 2012; Pot *et al.* 2014; Zheng *et al.* 2015a). Because *Pediococcus* spp. are integral to the genus *Lactobacillus*, *Lactobacillus* spp. and *Pediococcus* spp. were referred to as *Lactobacillus sensu lato* by Zheng *et al*. (2015). High resolution phylogenetic analyses also indicate that heterofermentative lactobacilli may be more closely related to species in the heterofermentative *Leuconostococcaceae* than to distant groups of homofermentative lactobacilli (Makarova *et al.* 2006; Sun *et al.* 2015; Zheng *et al.* 2015a) the term "*Lactobacillus* complex" was coined to encompass *Lactobacillaceae* and *Leuconostococcaceae* (Sun *et al*. 2015). **BOX ends.**

HABITATS OF LACTOBACILLI

Restricted by fastidious growth requirements, lactobacilli occupy nutrient-rich habitats which can be categorized into fermented or spoiled foods and animal feed, the environment including plants surface, soil, and the body of invertebrate and vertebrate animals (Fig. 1).

Food and feed. Lactobacilli dominate the microbiota of the vast majority of fermented foods and also occur as food spoilage organisms (Hammes and Hertel 2006; Gänzle 2015). Fermentation of silage, vegetables and many cereals relies on the microbiota of the raw materials as source of inoculum. Other fermentations, including most dairy fermentations, sourdough and fermented meats are controlled by back-slopping or a “house microbiota” associated with the production environment (Scheirlinck *et al.* 2009; Su *et al.* 2012; Chaillou *et al.* 2013; Ripari, Gänzle and Berardi 2016). Organisms in these fermentations are exposed to continuous propagation over decades or even centuries, essentially becoming domesticated to the fermentation environment (van de Guchte *et al.* 2006; Vogel *et al.* 2011; Ding *et al.* 2014). Adaptation to conditions in food fermentations was suggested for *L. delbrueckii* ssp. *bulgaricus*, which shows rapid and ongoing reduction of the genome size (van de Guchte *et al.* 2006). However, genomic analysis of intestinal and sourdough isolates of *L. reuteri* indicated differential selective pressure in the two environments but no phylogenetic differentiation (Zheng *et al.* 2015b). Therefore, even though the majority of the type strains have been isolated from food (Fig. 1a), food fermentations are unlikely to represent the primary habitat for *Lactobacillus* spp. (Fig. 1b).

Environmental sites and plants. Lactobacilli occur frequently in sewage as a result of fecal contamination and occasionally in soils as part of the rhizosphere of plants or as a result of wash off from the phyllosphere (Kvasnikov, Kovalenko and Nesterenko 1983; Hammes and Hertel 2006). Despite the occasional reports of lactobacilli being isolated from wheat, beet and strawberries (Jacobs, Bugbee and Gabrielson 1985; de Melo Pereira *et al.* 2012; Minervini *et al.* 2015), lactobacilli are a rare and minor component of the plant endophytes (Hallmann *et al.* 1997) and are only detected in small numbers on plant surfaces where traces of sugars can support their growth (Mercier and Lindow 2000). Their numbers only increase upon damage of plant tissue when simple and complex carbohydrates become available substrates (Müller and Lier 1994). The ecological role of plant-associated lactobacilli in nature is poorly understood, but because their occurrence is only sporadic, they are not considered plant symbionts but rather epiphytic (Stirling and Whittenbury 1963; Mundt and Hammer 1968; Fenton 1987).

Vertebrate and invertebrate hosts. Lactobacilli are reliably isolated from a variety of insects including flies and bees and from vertebrates, particularly birds, rodents, humans and farm animals. The host range is likely larger as scientific investigations have been largely restricted to domesticated animals and humans (Endo, Futagawa-Endo and Dicks 2010; McFrederick *et al.* 2013; Martino *et al.* 2016). Food storage organs such as the forestomach and crop appear to be the preferred habitat of lactobacilli in animal hosts. These organs are found in both insects (flies, bees, bumblebees) and vertebrate animals (poultry, rodents). In humans, lactobacilli are found in the oral cavity, gastrointestinal tract, and in the vagina (Walter 2008).

WHAT ARE THE REAL LIFESTYLES OF LACTOBACILLI IN NATURE AND HOW HAVE THEY EVOLVED?

Although we have a comprehensive knowledge of the origin of *Lactobacillus* strains, the precise ecological niches and lifestyles of these bacteria are difficult to unravel. To date, most functional research concerns the metabolic and, more recently, genetic adaptations to conditions that prevail in food and feed fermentations (Fig. 1a). However, although food fermentations provide opportunities for clonal expansion of specific species or phylogenetic groups (Cai *et al.* 2007; Chaillou *et al.* 2013; Zheng *et al.* 2015b), the adaptation of lactobacilli to these men-made habitats is coincidental and recent, and diversification, if it occurs, remains below the species level (Cai *et al.* 2007; Chaillou *et al.* 2013; Zheng *et al.* 2015b). From an evolutionary perspective, food, feed and biotechnological fermentations cannot be considered as habitats that supported speciation and therefore cannot inform the elucidation of the actual natural history of lactobacilli (Fig. 1b). Some species have been traced to animals, the environment, and raw materials (Scheirlinck *et al.* 2009; Su *et al.* 2012; Chaillou *et al.* 2013; Ripari, Gänzle and Berardi 2016), however, the real ecological niches of most *Lactobacillus* species present in food and feed remains unknown.

Predictions about the exact natural history of lactobacilli are difficult even for species that are reliably found in habitats that could support speciation. Lactobacilli can be 'allochthonous', meaning, they originate from a different place, and have, in contrast to 'autochthonous' species, neither an ecological nor evolutionary relationship with the habitat in which they are found. This is especially relevant for the gastrointestinal tract of humans where lactobacilli can originate from fermented food (Tannock 2004; Walter 2008, Fig.S1). It also relates to other habitats including wastewater, plants, flowers and nectar, where lactobacilli may be present as fecal contaminants from vertebrates or insects.

Autochthonous organisms establish stable populations of typical sizes over long periods and exert specific ecological functions in a habitat (Tannock 2004). However, even if populations show these characteristics, conclusions regarding the natural history of a species must be drawn with caution. The populations of allochthonous species may appear stable if introduced regularly into a habitat and they may exert ecological functions even if such habitats are irrelevant to their evolution, as is the case of fermented foods. On the other hand, habitats or hosts that only allow sporadic and transient colonization may still play an important role in the overall lifestyle of a species, for example by providing vectors for dispersal or a temporal refuge (Vellend 2010). It is also conceivable for some species to possess a dynamic lifestyle, comprised by more than one stable niche in which a classic autochthony could evolve.

Given these complexities, a combination of complementary approaches is required to reliably elucidate the natural history of lactobacilli. Below we attempt to deduce the lifestyles of *Lactobacillus* species by synthesizing phylogenomic data with information on the metabolism of the bacteria and inform these inferences with findings from more focused population genetic and functional studies. Specifically, we (*i*) assign lifestyles to species in a phylogenetic context, considering factors such as occurrence and frequency of detection/isolation as well as the strains' metabolic characteristics and their ability to withstand environmental stressors present in given habitats; (*ii*) investigate evolutionary transitions among lifestyles by using a phylogenetic approach that is conceptually similar to that described by Sachs and co-workers (Sachs, Skophammer and Regus 2011); (*iii*) analyze patterns of genome evolution described to be associated with the evolution of symbiotic lifestyles (Lo, Huang and Kuo 2016); (*iv*) complement this overview with findings from fine-scale population-genetic and functional studies on representative species that can serve as paradigms for the specific lifestyles represented within the lactobacilli.

Evolutionary insight through phylogenomics

The diversification of anaerobic clostridia and aerobic or facultative anaerobic bacilli and the lactic acid bacteria roughly matches the "great oxidation event" that occurred ~2.5 billion years ago (Battistuzzi *et al.* 2004). *Lactobacillales* then diverged from staphylococci and bacilli approximately 1.8 billion years ago (Battistuzzi *et al.* 2004), substantially predating the emergence of land plants (~500 million years ago), insects (~400 million years ago), mammals (~200 million years ago) and birds (~80 million years ago) (Shetty, Griffin and Graves 1999; Hedges *et al.* 2004; Luo 2007; Clarke, Warnock and Donoghue 2011; Pires and Dolan 2012; Misof *et al.* 2014). However, diversification within the genus *Lactobacillus sensu lato* likely intensified with the emergence and later diversification of the eukaryotic species with which lactobacilli became associated (Tailliez 2001)

To gain insight into lifestyle evolution of lactobacilli, we updated the core phylogenomic tree of *Lactobacillus sensu lato* (Zheng *et al.* 2015a) by adding species for which genome sequences became recently available (Fig. 2). Based on isolation source, frequency of isolation, metabolic capabilities, growth temperature and the ability to withstand environmental stressors present in given habitats, we assign species into three main lifestyle categories: free-living (encompassing environmental and plant isolates), host-adapted (associated with invertebrate or vertebrate hosts), or as 'nomadic' using the concepts proposed by Martino and co-workers

(Martino *et al.* 2016). Remarkably, lifestyle assignments associate with phylogenetic grouping (Fig. 2). This strongly suggests that monophyletic clades within the lactobacilli result from adaptive evolution in different habitats, which resulted in the emergence of distinct lifestyles and a high degree of phylogenetic niche conservation. Specifically, the *L. perolens*, *L. sakei*, *L. vaccinostercus, L. collinoides*, *L. brevis* and *L. buchneri* groups are almost completely composed of species that are rarely found in animals and are therefore likely free-living. Species in the *L. reuteri* group are consistently associated with vertebrate hosts (human oral cavity, vagina, and intestinal tract, primates, and other mammals and birds). The *L. salivarius* group contains a monophyletic cluster associated with vertebrate hosts (humans, rodents, birds, horses, cattle, swine, primates and other mammals) (Table S1) and a second cluster comprising mainly free-living species. The large and diverse *L. delbrueckii* group comprises a cluster of species adapted to insects and another adapted to vertebrates including pigs and hamsters and different species of birds. Species in the *L. plantarum* group and a cluster with the *L. casei* group are nomadic, being reliably found in a wide variety of niches.

The conservation in the niche assignments within the deep-branching monophyletic lineages suggests that lifestyles often evolved for long periods of evolutionary time and were stably maintained. These clear associations further allow to pinpoint lifestyle transitions, which occurred in 8 separate events (See Fig.2 and legend for details). The host adapted *L. delbrueckii, L. salivarius* and *L. reuteri* groups likely evolved from free-living ancestors to become associated with vertebrates (events 1-3), while the *L. fructivorans*, *L. kunkeei* and *L. mellifer* groups became associated with insects (events 4 and 5). Within the *L. delbrueckii* group, the cluster of species related to *L. apis* appeared to have switched hosts and transitioned from vertebrate-adapted to bee-adapted (event 6). The *L. plantarum* group and a cluster within *L. casei* group evolved from free-living to nomadic (events 7 and 8). *L. fermentum* is the only species in the *L. reuteri* group which is rarely found in intestinal ecosystems but frequently isolated from plants and spontaneously fermented cereals (Mundt and Hammer 1968; Hammes and Hertel 2006; Gänzle and Ripari 2016). *L. fermentum* could be an example of a species undergoing reversion of the lifestyle from host-adapted to free-living, a process that has been documented for environmental species that cluster within phylogenetic clades dominated by symbionts (Sachs, Skophammer and Regus 2011).

Patterns of genome evolution reflect an evolutionary transition to a symbiotic lifestyle

The genomes of lactobacilli range in size from 1.27 (*L. iners*) to 4.91 (*L. parakefiri*) Mb and the number of genes between species varies considerably (Sun *et al.* 2015, Table S1). Lactobacilli underwent a process of genome reduction over the course of their evolution, losing on average 3000 genes from the common ancestor and 1,300–1,800 genes in individual groups or species (Makarova *et al.* 2006; Sun *et al.* 2015; Zheng *et al.* 2015a). Gene decay in lactobacilli has led to substantial loss of functions in carbohydrate metabolism, amino acid and cofactor biosynthesis, leading to the fastidious nutritional requirements of the species (Makarova *et al.* 2006). This process is especially pronounced in lactobacilli associated with animals (Sun *et al.* 2015) and has been attributed to the nutrient-rich environment found within host habitats (Makarova *et al.* 2006). However, genome reduction is an evolutionary process that is universally observed in symbionts and directly associated with the degree of host specialization (Lo, Huang and Kuo 2016). The stable environment provided by the host renders functions that were essential in the free-living ancestor superfluous, which leads to an accumulation of loss-of-function mutations and pseudogenes followed by removal of these genetic regions, e.g. through mobile genetic elements (Lo, Huang and Kuo 2016). Genome reduction is strongly correlated with host adaptation in *Lactobacillus* species, with genome size being significantly lower in host-adapted compared to nomadic and free-living species (Fig. 3a and b). Interestingly, genomes of host-adapted lactobacilli also show a reduction in GC content, while reduction of GC content is not observed in nomadic lactobacilli (Fig. 3c and d). This constitutes another well documented pattern observed in the genome evolution of host-adapted symbionts and is caused by nonadaptive loss of DNA repair genes followed by a strong mutational bias toward A (adenine) and T (thymine) (Lo, Huang and Kuo 2016). Taken together, host-association in lactobacilli is associated with genomic events that are characteristic of the evolution of a symbiotic lifestyle.

Metabolic capabilities reflect lifestyle adaptations

Species within the *Lactobacillus sensu lato* show a substantial degree of variation in their metabolism (Gänzle 2015; see Box 2 for a detailed description). The two major physiological groups, homofermentative and heterofermentative organisms, differ not only with respect for the metabolic pathway used for hexose fermentation but also in the presence or absence of other major metabolic pathways for carbohydrate and amino acid utilization. These two physiological groups also represent two separate phylogenetic clades of within the genus *Lactobacillus* sensu lato (Fig. 2; Gänzle, 2015; Zheng et al., 2015). Both homo- and heterofermentative species associate with vertebrate animals, insects or environmental habitats (Fig. 2), however, metabolic adaptation to the diverse lifestyles is observed at the group (genus) level (Zheng et al., 2015). Remarkably, many habitats harbour homofermentative and heterofermentative *Lactobacillus* species that co-exist. Examples include intestinal habitats such as the gut microbiota of fruit flies (*L. plantarum* and *L. fructivorans* groups), bees (*L. mellifer* or *L. delbrueckii* and *L. kunkeii* groups)(Anderson *et al.* 2013; Filannino *et al.* 2016) and vertebrate animals (*L. delbrueckii* and *L. reuteri* groups)(Walter 2008) as well as fermentation or spoilage microbiota in many foods including cereal, vegetable and meat (Gänzle 2015; Hammes and Hertel 2006). This coexistence supports emerging evidence that indicates that homo- and heterofermentative lifestyles are complementary rather than competitive (Gänzle, Vermeulen and Vogel 2007; Tannock *et al.* 2012; Andreevskaya *et al.* 2016; Andreevskaya 2017).

Additional differences in carbohydrate utilization patterns and growth temperature provide helpful insights into niche adaptations. Free-living species are consistently capable of growing at lower temperatures, while host-adapted species grow optimally at temperatures close to the body temperature of their corresponding hosts (Fig. 3e). The enzymatic repertoire of the species is also indicative of the substrates available in their natural habitats (Zheng et al., 2015a). Below we use this information to elucidate the exact lifestyle of the species and the characteristics of the niches to which they have adapted to.

BOX 2. Metabolism of lactobacilli. Lactobacilli are oxygen-tolerant or anaerobic and grow at temperatures ranging from 2 to 53 °C (Pot *et al.* 2014). Although many species retain the capacity for conditional respiration (Brooijmans, de Vos and Hugenholtz 2009; Zotta *et al.* 2016), lactobacilli predominantly derive metabolic energy through substrate level phosphorylation (Gänzle 2015), abandoning the metabolic efficiencies of either anaerobic or aerobic electron transport chains (Pedersen *et al.* 2012). This “selfish” metabolic behavior of lactobacilli, which is also exhibited by the yeast *Saccharomyces*, reflects adaptation to nutrient-rich environments (Spor *et al.* 2009; Mora, Arioli and Compagno 2013). The adaptation to nutrient-rich habitats is also reflected by the multiple auxotrophies for amino acids, nucleotides and vitamins (Ricciardi *et al.* 2015). Notwithstanding the rare presence of extracellular amylases, inulinases, xylanases (Yong, Lee and Hutkins 2007; Gänzle and Follador 2012; Pontonio *et al.* 2016), extracellular glycosyl hydrolases are typically absent in lactobacilli and carbohydrate metabolism relies almost exclusively on oligo- or monosaccharide transport and intracellular metabolism (Gänzle and Follador 2012). Lactobacilli are in general auxotrophic for essential amino acids but most strains possess proteolytic systems to hydrolyze protein sources available in their nutrient-rich habitats (Savijoki, Ingmer and Varmanen 2006).

The *Lactobacillus sensu lato* is separated into two major metabolic groups representing homo- and heterofermentative lactobacilli; organisms representing these two groups also cluster in separate phylogenetic clades (Fig. 2, Gänzle, 20115ö Zheng *et al.* 2015a). Homofermentative lactobacilli metabolize hexoses via the Embden-Meyerhof Pathway, yielding two moles of lactate and two moles of ATP per mole of glucose. Heterofermentative lactobacilli lack the gene coding for phosphofructokinase (Zheng et al., 2015a) and metabolize hexoses via the phosphoketolase pathway, yielding lactate, ethanol, CO_2 and only one mole of ATP per mole of glucose unless electron acceptors are available (Gänzle 2015). The ecological fitness of heterofermentative lactobacilli depends on the preferred utilization of disaccharides, the simultaneous utilization of pentoses and hexoses, and preferential utilization of fructose, phenolic acids, and a broad range of aldehydes as electron acceptor (Gänzle, Vermeulen and Vogel 2007; Gänzle 2015; Andreevskaya *et al.* 2016; Filannino *et al.* 2016). Genetic differences between homo- and heterofermentative lactobacilli not only relate to key enzymes of the Embden-Meyerhof and Phosphoketolase pathways but also relate to differential preference for metabolic pathways that are used for lactose- and fructose metabolism, and include the loss of genes related to carbohydrate metabolism and transport, particularly PTS systems, and pyruvate formate lyase in heterofermentative lactobacilli (Gänzle et al., 2015; Zheng *et al.* 2015a). The agmatine deiminase pathway and ornithine decarboxylase employ products of bacterial arginine conversion by decarboxylation or the arginine deiminase pathway as substrates and thus support trophic relationships between bacteria. These pathways increase acid resistance and are predominantly but not exclusively found in hetero and homofermentative organisms, respectively. **End of BOX.**

PARADIGMS OF *LACTOBACILLUS* LIFESTYLES

Free-living lifestyle

Species that are found in plant and environmental sources are scattered around the phylogenetic tree (Fig. 2), which suggests a free-living common ancestor of the genus. Free-living lactobacilli are clustered in the *L. buchneri* and *L. collinoides* groups and constitute all the species in the *L. brevis, L. composti and L. perolens* groups (Fig. 2).

Although it is difficult to determine if a lifestyle is strictly free-living, this is strongly suggested by several characteristics of organisms in these clades. First, species within the phylogenetic groups are mostly isolated from plants or fermented plant products and very rarely from animals (Mundt and Hammer 1968; Daeschel, Andersson and Fleming 1987). Second, the metabolic and physiological properties of the strains are reflective of a free-living lifestyle, with most species being aerotolerant by using a Mn (II) defense mechanism against oxygen toxicity (Daeschel, Andersson and Fleming 1987). Additionally, their optimal growth temperature is closer to temperatures of terrestrial and aquatic habitats as most species are able to grow at 15°C but not at 45°C (Table S1 Fig. 3f). Third, they possess large genomes (Fig. 3a and b) encoding a versatile range of enzymes to utilize a wide spectrum of substrates, including pentoses, sucrose, lactose, mannitol, melezitose, cellobiose, nitrate, citric acid and malic acid (Danner *et al.* 2003; Zheng *et al.* 2015a; Martino *et al.* 2016). Pentoses that are liberated upon degradation of plant materials as a result of hydrolysis of hemicellulose (Dewar, McDonald and Whittenbury 1963) are utilized by free-living lactobacilli through the pentose phosphate or phospoketolase pathways (Gänzle 2015). Interestingly, the ability to ferment pentoses is rarely found in yeasts, suggesting a possible mechanism of niche partition between lactobacilli and yeasts in their shared natural habitats (Mundt and Hammer 1968), which may be key to the success of lactobacilli in nature. Two species that fit all the three criteria above are the psychrotrophic *L. hokkaidonensis* (Tohno *et al.* 2013, Tanizawa *et al*. 2015) as well as *L. buchneri* (Heinl *et al*. 2012). These species are isolated from grass silage, are aero-tolerant, have a preference for pentose over hexose metabolism, can grow at 15 °C, and have genomes of >2.3 kb (Tanizawa *et al*. 2015,Table 1).

Host-adapted lifestyle

The ability to colonize eukaryotic hosts benefits lactobacilli for several reasons; (*i*) their fastidious requirements for nutrients are satisfied in several host-associated niches; (*ii*) they often share the same food sources as their hosts (plants, fruits, nectar, etc. rich in carbohydrates, amino acids, vitamins, and nucleotides); and (*iii*) they can use host animals as vectors to migrate to new habitats (Hammes and Hertel 2006; Mundt and Hammer 1968). Lactobacilli are found in vertebrates and insects, but as described above, not all species found are autochthonous, and even those that are differ markedly in the degree of specificity towards particular hosts or body sites. Examples are listed in Table 1. Below we discuss the research on representative species that can serve as paradigms for host-associated lifestyles in lactobacilli.

Lactobacilli adapted to vertebrate hosts. Species that colonize vertebrate hosts cluster within the monophyletic *L. delbrueckii, L. salivarius*, and *L. reuteri* groups which are predominantly composed of host-associated species. This suggests that the vertebrate-associated lifestyle is the outcome of a long-term evolutionary process that brought about a stable co-existence with vertebrate animals. However, lineages did not remain within specific host species and the members of the phylogenetic groups differ in terms of host range, colonization site (gut, oral cavity, vagina) and the degree of specialization. This indicates that following initial adaptation to vertebrate hosts, further diversification and specialization occurred at the species level. Among the species for which the vertebrate lifestyle is best understood are *L. reuteri*, *L. ruminis*, *L. salivarius*, *L johnsonii, L. amylovorus* and *L. iners* (Table 1). With exception of *L. iners*, these species are found in the oral cavity and digestive tracts, and a number of characteristics reflect their adaptation to the gut. They tolerate bile acids, are highly acid-resistant and ferment oligo- and polysaccharides present in the diet of their hosts (Kakimoto *et al.* 1989; Grill *et al.* 2000; Lähteinen *et al.* 2010; Gänzle and Follador 2012; Ruiz, Margolles and Sánchez 2013; O' Donnell *et al.* 2015; Zheng *et al.* 2015a; Krumbeck *et al.* 2016). Additionally, these species grow optimally at 37°C and higher (Table 1), which reflects the body temperatures of most mammals and birds.

Vertebrate-associated lactobacilli typically colonize a range of host species. Exceptions include the human vaginal species *L. jensenii* and *L. iners* and the pig-associated *L. amylovorus*. *L. amylovorus* is a dominant member of the porcine microbiota (Leser *et al.* 2002; Konstantinov *et al.* 2004, 2006; Marti *et al.* 2010; Chang *et al.* 2011; Kant *et al.* 2011) but is rarely detected in other animals (Nakamura 1981; Reti *et al.* 2013; Guan *et al.* 2003; Hammes and Hertel 2006), suggesting that it is host-specific to pigs. The species dominates the microbiota on the *pars non-glandularis* region of the pig stomach, which is characterized by a dense biofilm composed of lactobacilli (Pedersen and Tannock 1989; Mann *et al.* 2014). In addition, *L. amylovorus* is one of few lactobacilli capable of utilizing amylose by the extracellular hydrolysis of starch (Gänzle and Follador 2012), a trait that is likely to contribute to the ecological fitness of the species in the distal intestinal tract of pigs (Regmi *et al.* 2011).

The highest degree of niche specialization in vertebrate-adapted lactobacilli occurs in the human vagina. The vaginal microbiota is dominated by *L. iners*, *L. crispatus*, *L. jensenii* and *L. gasseri* (Anderson *et al.* 2014; Mendes-Soares *et al.* 2014). *L. jensenii* and *L. iners* are only found in this niche, and the latter species shows the highest degree of specialization observed among the currently known lactobacilli. *L. iners* has the smallest genome of all known lactobacilli and more complex nutritional requirements, reflected by its inability to grow on standard growth media (Macklaim *et al.* 2011; Petrova *et al.* 2016). It appears that the species has evolved an almost obligate symbiotic lifestyle highly dependent on the human host. The presence of specific genes, such as the Fe-S protein cluster, enables defense against oxidative stress from H_2O_2 produced by other vaginal lactobacilli (Macklaim *et al.* 2011). Although biofilms are normally not observed in the healthy vagina, host specificity of *L. iners* is likely achieved by specific adherence cells in the vaginal epithelium (Fig.4a, Macklaim *et al.* 2011).

The species *L. reuteri*, *L. ruminis*, *L. johnsonii*, *L. salivarius*, *L. cripatus*, *L. acidophilus* and *L. vaginalis* have a broader host range and are found in different body sites (Table 1). However, the population structure of *L. reuteri*, *L. ruminis*, and *L. johnsonii* indicates that subpopulations within these species adapted and specialized to particular host animals. All three species separate in phylogenetic clusters that are highly reflective of host origin (Oh *et al.* 2010; Buhnik-Rosenblau *et al.* 2012; O' Donnell *et al.* 2015). For *L. reuteri*, these clusters have been established by Amplified Fragment Length Polymorphism, Multilocus Sequence Analysis (Oh *et al.* 2010 Fig. 5a) and whole genome phylogenies (Wegmann *et al.* 2015; Duar *et al.* 2017). Additionally, the genome content of strains from different phylogenetic clusters is reflective of the niche characteristics in respective hosts (Frese *et al.* 2011). *L. reuteri* is regarded as autochthonous to the human gut (Reuter 2001) and has been found to be a prevalent member of the microbiota of traditional agriculturalist societies (Martínez *et al.* 2015). The genomes of human strains of *L. reuteri* are characterized by a closed pangenome (a limited maximum number of genes in their gene pool not much larger than that of a single strain) and extensive deletion of large adhesin-like surface proteins, but conserve the ability to utilize glycerol and propanediol as electron acceptors, suggesting growth in the intestinal lumen (Frese *et al.* 2011; Walter, Britton and Roos 2011). In contrast, rodent *L. reuteri* strains possess an open pangenome (a large gene repertoire vastly exceeding this of a single strain) and several large adhesin-like surface proteins and colonize by adhering to the surface of the squamous stratified epithelia of the forestomach of mice on which they form biofilms (Walter *et al.* 2005, 2007; Frese *et al.* 2013; Fig. 4b). These findings do not only indicate a tight interrelationships between *L. reuteri* and particular hosts during their joint evolution, but also fundamentally different paths of genome evolution in different host-adapted lineages.

L. reuteri has further been established as a model species to study mechanisms of host adaptation in lactobacilli (Walter, Britton and Roos 2011; Kwong and Moran 2015). Host specificity has been experimentally demonstrated in competition experiments in gnotobiotic mice and more recently in chickens (Oh *et al.* 2010; Frese *et al.* 2011; Duar *et al.* 2017). *L.*

reuteri isolated from both rats and mice cluster together in phylogenetic lineages and, accordingly, rat isolates are very competitive in mice. Similarly, isolates from chicken and turkeys group in the same phylogenetic lineages (Oh *et al.* 2010; Frese *et al.* 2011; Duar *et al.* 2017). These findings demonstrate that *L. reuteri* has adapted to groups of related hosts, such as rodents or species of poultry that possess similar niches in their intestinal tracts and whose social behavior allows horizontal transfer of bacteria (Oh *et al.* 2010). Functional studies with loss-of-function mutations have demonstrated that the ecological success of rodent strains in the forestomach depends on biofilm formation (Fig. 4b) and resistance to gastric acidity (Walter *et al.* 2007; Frese *et al.* 2013; Krumbeck *et al.* 2016). Inactivation of one single serine-rich surface adhesin specific to rodent strains with a devoted transport system (the SecA2-SecY2 pathway) completely abrogated biofilm formation, indicating that initial adhesion represents the most significant mechanism underlying host-specific colonization (Frese *et al.* 2013).

Similar mechanistic studies are lacking in other species of lactobacilli but comparable genomic patterns of host adaptation are observed, e.g. for *L. ruminis*. Human isolates of *L. ruminis* are aflagellate and non-motile while bovine, equine and porcine isolates are motile, with the latter two being hyper-flagellated (O'Donnell *et al.* 2015). These differences in the expression of flagella and motility may reflect adaptation to the conditions in different hosts, although this remains to be established experimentally. Overall, the data available for *L. reuteri* and *L. ruminis* indicate that some lactobacilli evolved a high degree of host-specialization. Moreover, robust clustering in defined phylogenetic groups based on host origin indicates that these host associations are maintained over evolutionary timescales. Finally, the high fidelity in epithelial recognition for biofilm formation of bacterial strains, as demonstrated for *L. reuteri* (Frese *et al.* 2013), providing a mechanism by which lineages are reliably transmitted from generation to generation and maintained over both ecological and evolutionary time scales.

Other host-adapted species appear to have more 'promiscuous' lifestyles, both in terms of host range and body site. *L. salivarius* is indigenous to the human oral cavity (Rogosa *et al.* 1953) and is one of few *Lactobacillus* species that has been consistently recovered from the feces of human individuals for at least 18 months (Tannock *et al.* 2000). *L. salivarius* has also been obtained from breast milk (Martín *et al.* 2006) and a variety of body sites including the intestinal mucosa (Molin *et al.* 1993), tongue, rectum (Ahrné *et al.* 1998) and the vagina (Vera Pingitore *et al.* 2009). This species is found in pigs (Mackenzie *et al.* 2014), chicken (Hammons *et al.* 2010) hamsters (Rogosa *et al.* 1953) and horses (Yuki *et al.* 2000). Phylogenetic analysis of strains from a variety of sources did not show clustering by origin, but many isolates show signs of ongoing adaptation by genome decay (Raftis *et al.* 2011), indicating an ongoing process of specialization. *L. vaginalis* and *L. gasseri* can be detected in oral and fecal microbiota of the same species (Dal Bello and Hertel 2006) and they are also members of the vaginal microbiota. Therefore, it appears that these species maintain more dynamic and flexible lifestyles regarding host range and ecological niche in comparison to *L. reuteri* and *L. ruminis*.

Lactobacilli associated with invertebrate hosts. The association of lactobacilli with invertebrates is a more recent discovery (Engel and Moran 2013; Shrivastava 1982). Insect-associated species are distributed across the *Lactobacillus* phylogeny (Fig. 2) and cluster in phylogenetic groups with different levels of host specificity (McFrederick *et al.* 2013). Species associated with bees cluster in the *L. kunkeei* and *L. mellifer* groups and in the *L. helsinborgensis* clade of the *L. delbrueckii* group (Fig. 2), which were termed as the Firm 4 and Firm 5 phylotypes prior to description of the species (Ellegaard *et al.* 2015). This finding suggests that association with bees occurred in independent events (events 6 and 4, Fig. 2). Species of the *L. fructivorans* group (Fig. 2) are also often associated with insects but appear to be transmitted between host species by floral transmission (McFrederick *et al.* 2012).

Species belonging to all four groups have small genomes (Zheng *et al.* 2015a; Maeno *et al.* 2016; Fig. 2, Table 1) and extremely limited carbohydrate fermentation capabilities (Ellegaard *et* *al.* 2015), being essentially restricted to a "sucrose and maltose diet". Heterofermentative lactobacilli associated with bees are fructophilic, they lack alcohol dehydrogenase activity and depend on the availability of fructose as electron acceptor (Endo, Futagawa-Endo and Dicks 2009; Filannino *et al.* 2016; Maeno *et al.* 2016). It is likely that these restrictions reflect adaptations not only to the host's diet (i.e. honey, nectar and pollen for bees) but also the differences in the competitive interactions that occur within the gut environments. Compared to vertebrates, bees harbor relatively simple microbial communities composed of nine bacterial species clusters and there is compelling evidence that species occupy distinct and complementary metabolic niches within the bee gut (Powell *et al.* 2016). Therefore, specialization as a means of niche partitioning and syntrophic interaction seems to be one of the key mechanisms to the ecological success of bee-associated lactobacilli species (Kwong and Moran 2016).

Lactobacillus species are often dominant members of the microbiota of some species of Hymenoptera (ants, bees and wasps) (Kwong and Moran 2016). However, only honey and bumble bees have been described to date to harbor host-specific lineages of lactobacilli (McFrederick *et al.* 2013). Both the *L. mellifer* group and *L. helsinborgensis* clade are almost ubiquitously represented in individual bees, they are particularly abundant in adult workers and the queen bee, with individual lineages being specific to honey and bumble bees (Vásquez *et al.* 2012; Kwong and Moran 2016). Species within these clades are oxygen-sensitive and have not been found outside the bee gut, and are therefore likely obligate symbionts colonizing the anoxic regions of the distal hindgut, which is also supported by the genomic signatures of these species. All species have small genomes, (< 2.1 Mb) with low GC contents ranging from 34.6 to a 36.6%, and most strains can grow at 15 °C and optimally at temperatures significantly lower than those adapted to vertebrates (Fig. 3e, Table S1).

The *L. kunkeei* group are dominant members in the crop microbiota of bees and the species *L.*

kunkeei is a major component of the biofilm that is found in the bee crop as determined by 16S rRNA sequencing (Vásquez *et al.* 2012; Fig. 4c). However, the species can also be detected in pollen, nectar and hive materials, as well as from fresh flowers and fruits (Endo *et al.* 2012; Neveling, Endo and Dicks 2012; Anderson *et al.* 2013), and it apears to migrates frequently between honey bees and stingless bees, suggesting that the species is more 'promiscuous' than the host-specific members of the *L. mellifer* group and *L. helsinborgensis* clade (Tamarit *et al.* 2015). *L. kunkeei* is obligately fructophilic*,* osmotolerant, resistant to high concentration of phenolic acids present in pollen and utilizes phenolic acids as electron acceptors (Filannino *et al.* 2016). Moreover, it resists the antimicrobial activity of royal jelly (Vojvodic *et al.* 2013) and the desiccant conditions in honey (Endo *et al.* 2012; Vojvodic *et al.* 2013). The exact role of the environmental niches in the lifestyle of *L. kunkeei* is unclear. The species might be able to stably colonize fruits and flowers and/or use them for transmission. Alternatively, *L. kunkeei* might just be an allochtnonous contaminant of fruits and flowers that gets deposited at these sites during bee pollination and foraging (McFrederick *et al.* 2012; Tamarit *et al.* 2015).

Species in the *L. fructivorans* group are shared between plants and insects including sweat bees and *Drosophila*. From its six species of the group two were isolated from insects (*L. fructivorans*, *L. vespulae*), two from flowers (*L. ixorae*, *L. florum*) and two (*L. sanfranciscensis* and *L. homohiochii*) from fermented food products (Kitara, Kaneko and Goto 1957; Endo *et al.* 2010; Vogel *et al.* 2011; Wong, Ng and Douglas 2011; McFrederick *et al.* 2013; Techo *et al.* 2016, Fig. S1). Although their lifestyle has not yet been studied, their reduced genome size (<1.5 Mb, with *L. sanfransciscensis* possessing one of the smallest genomes of all lactobacilli) and low GC content (Fig. 3a - d) display the classic hallmarks of symbiosis (Lo, Huang and Kuo 2016). We therefore consider this group 'insect-adapted', but more research is needed to elucidate the exact lifestyle of its members.

Contrary to bees, *Drosophila* species do not harbor a defined core-microbiota and the composition varies widely between individual colonies and among populations in the wild and in laboratories. In fact, the microbiota of fruit flies is composed mainly of *Acetobacte*r and *Lactobacillus* species that are also found in other habitats, including the environment, dairy and vertebrate animals (Chandler *et al.* 2011; Wong, Ng and Douglas 2011; Erkosar *et al.* 2013; Wong, Chaston and Douglas 2013), and diet plays a major role in shaping the microbiome of *Drosophila* spp. (Wong *et al.* 2015). It appears therefore that the association of lactobacilli with fruit flies is less host-restricted and more dynamic than the insects discussed above, with an important impact of the immediate environment of the insects (Wong *et al.* 2015). Such a lifestyle can be considered ‘nomadic’.

“Nomadic” species of lactobacilli

Most of the *Lactobacillus* species found in the human gut do not form stable populations and have been categorized as allochthonous as they are derived from food or feed (Tannock *et al.* 2000; Walter *et al.* 2001; Tannock 2004; Walter 2008). However, although not autochthonous in the classical sense, some *Lactobacillus* species, such as *Lactobacillus plantarum*, *Lactobacillus casei, Lactobacillus paracasei* and *L. rhamnosus* possess adaptations to gut ecosystems and the oral cavity that allow them to persist for at least a limited time (Table 1). These species possess large genomes with little evidence for specialization to particular habitats and they are found in invertebrate hosts, different body parts of vertebrates (i.e. gut, oral cavity, vagina), and in food materials, such as meat, fish, vegetables and raw or fermented dairy products (Kandler and Weiss 1986; Stiles and Holzapfel 1997; Heilig *et al.* 2002; Wall *et al.* 2007; Delgado, Suárez and Mayo 2010; Siezen *et al.* 2010; Ceapa *et al.* 2016; Rossi *et al.* 2016). Recent research on *L. plantarum* has provided convincing evidence that the species represent a paradigm for a nomadic lifestyle (Martino *et al.* 2016).

Nomadic *Lactobacillus* species cluster in two phylogenetic groups, the *L. plantarum* group and a cluster within the *L. casei* group (Fig. 2). Like free-living lactobacilli, their large genomes correspond to increased metabolic flexibility, and *L. plantarum* and *L. casei* retained the capacity for conditional respiration (Brooijmans, de Vos and Hugenholtz 2009; Zotta *et al.* 2016). In addition, *L. plantarum* WCFS1 encodes a large spectrum of sugar uptake and utilization cassettes, allowing the organism to grow on numerous carbon sources (e.g. plant oligo and polysaccharides), facilitating flexibility in different habitats (Siezen and van Hylckama Vlieg 2011). *L. casei* ATCC 393, a strain isolated from cheese, lacks the biosynthetic pathway for the branched chain amino acids valine, leucine and isoleucine but is capable of synthetizing all other amino acids except is thus able to thrive in protein-limited environments. The species also utilizes a great variety of carbohydrates (Cai *et al.* 2007) and contains 16 two-component systems, the highest number observed among lactobacilli, suggesting that gene expression is adjusted to changing environments (Cai *et al.* 2009).

The high genetic and phenotypic diversity of *L. plantarum*, *L. casei,* and *L. rhamnosus* has been established by both phenotypic and genotypic approaches (Bringel, Curk and Hubert 1996; Torriani *et al.* 2001; De Las Rivas *et al.* 2005; Molenaar *et al.* 2005; Cai *et al.* 2007, 2009; Diancourt *et al.* 2007; Siezen *et al.* 2010; Broadbent *et al.* 2012; Smokvina *et al.* 2013; Ceapa *et al.* 2015, 2016; Martino *et al.* 2016). Comparative genomic analysis of 54 *L. plantarum* strains demonstrated the absence of environmental specialization (Martino *et al.* 2016; Fig. 5b) which had been already hypothesized in previous studies (Molenaar *et al.* 2005; Siezen *et al.* 2010). As shown in Fig. 5b, *L. plantarum* strains display no obvious clustering by origin, with isolates from the same source being scattered throughout the tree. This in in stark contrast to the phylogenetic structure of strains of the host-adapted species *L. reuteri,* which cluster cohesively by host origin (Fig. 5a and b). Genes involved in exopolysaccharide biosynthesis, sugar metabolism and the secretome showed the most variability amongst *L. plantarum* strains but did not relate to specialization to any specific habitats (Martino *et al.* 2016). Similarly, *L. paracasei*, *L. casei*, and *L. rhamnosus* did not show a correlation between the habitat and phylogenetic position as determined by core and pan-genome phylogenies coupled with analyses of variable regions (Cai *et al.* 2009; Smokvina *et al.* 2013; Douillard *et al.* 2013).

L. plantarum, *L. casei* and *L. rhamnosus* do not form stable population in animal hosts but possess adaptive features to niches associated with humans and animals that contribute to their persistence. For example, several *L. plantarum* strains show high tolerance to gastric juice and bile acids (Bron *et al.* 2004b; van den Nieuwboer *et al.* 2016). *L. casei* adheres to intestinal villi (Galdeano and Perdigón 2004) and several strains of both *L. casei* and *L. paracasei* resist bile (Alcántara and Zúñiga 2012; Wang *et al.* 2010). *L. rhamnosus* possesses mucus-binding pili that might interact with the host epithelia in the oral cavity and the small intestine (Kankainen *et al.* 2009) that are present in a larger subset of strains(Douillard *et al.* 2013). *L. plantarum* WCFS1 responds to the gastrointestinal environment of mice by regulating a large array of genes (Bron *et al.* 2004a). Interestingly, persistence of *L. plantarum* in the gastrointestinal tract of mice increases after only three passages, which was accompanied by mutation acquisitions (van Bokhorst-van de Veen *et al.* 2013). These studies suggest that some *Lactobacillus* species can rapidly adapt to intestinal ecosystems and temporarily persist despite not being autochthonous members of the resident microbiota.

Taken together, evidence indicates that some *Lactobacillus* species have evolved a nomadic lifestyle that exerts diverse selective pressures rather than promoting niche specialization. Genomic and phenotypic characteristics of strains of these species appear unrelated to the origin of isolation, which highlights their ability to migrate across environments which is in line with their ubiquitous presence and their ability to thrive on various substrates. This feature could constitute a strategy of dissemination, or from an ecological perspective, dispersal (Vellend 2010). During evolution, these species, originally associated with plants, may have developed the ability to inhabit the gut of animals feeding on plants, favoring dissemination to new habitats. Dispersal influences the dynamics, composition and structure of communities and the distribution and abundance of species. From an evolutionary perspective, it affects processes such as local adaptation, speciation and the evolution of traits that ultimately impact the natural history of species (Dieckmann, O'Hara and Weisser 1999). Therefore, nomadic *Lactobacillus* species could have evolved dispersal traits in the form of colonization factors of host animals, allowing these immotile bacterial species to disseminate. Nomadic lifestyles of lactobacilli have also been identified in insects such as some species of Hymenoptera (sweat bees and ants) and fruit flies, (McFrederick *et al.* 2013; Matos and Leulier 2014) which represent excellent vectors for dissemination for bacteria that have their main habitat in plants and fruits. However, the lifecycle of some lactobacilli might even be more complex and dynamic, beginning with the excretion via feces, followed by mechanical distribution to and among plants and return to the host via the oral and alimentary cavity, as suggested in 1968 by Mundt and Hammer (Mundt and Hammer 1968). Future studies should be directed to reconstructing the natural and evolutionary history of nomadic lactobacilli in both vertebrates and invertebrates order to better understand their adaptation process and the relative dependence on free-living and host associated niches.

A hypothetical framework for the evolution of lifestyle transitions in the genus *Lactobacillus sensu lato*

The synthesis of phylogenomic, metabolic and functional data presented above provides a consistent view on the evolution of distinct lifestyles of lactobacilli (Fig. 6). A free-living ancestry for the *Lactobacillus sensu lato* is logical as symbioses with plants have not been described and the diversification from the bacilli predates the emergence of animals. From the ancestral state, the genus has diversified and evolved lifestyles that cover the entire spectrum from free-living to strictly host-adapted, with a substantial variation in the reliance on environmental niches and the degree of host-specificity.

The phylogenomic data supports a model by which *Lactobacillus* lineages have diversified and evolved symbiotic lifestyles on five separate occasions (event 1-5 in Fig. 2), resulting in the *L. delbrueckii, L. salivarius*, *L. reuteri*, *L. mellifer* and *L. kunkeei*/*L. fructivorans* phylogenetic groups. This evolutionary process is reflected by adaptations to the host environment (bile and acid tolerance, growth at host body temperature, metabolic adaptations to insects) and genomic changes (genome decay, decreased GC content, loss of biosynthetic enzymes) consistent with those found in other bacterial symbionts (Lo, Huang and Kuo 2016). Host-adapted lactobacilli differ in the degree of niche specialization and host dependence, ranging from 'promiscuous' to completely host restricted, with *L. iners* representing the most extreme cultural representative. Selective epithelial adhesion (often followed by the formation of biofilms) appear to be a key mechanism by which lactobacilli maintain stable associations with hosts over evolutionary times, as most animal sites with highly adapted species are characterized by adherent cells, e.g. the vagina, the crop of insects and birds, the forestomach of rodents and the non-glandular region of the stomach of pigs and horses (Fuller and Brooker 1974; Pedersen and Tannock 1989; Tannock 1992; Yuki *et al.* 2000; Vásquez *et al.* 2012; Frese *et al.* 2013; Mann *et al.* 2014).

Host-adapted lifestyles likely evolved after ancestral plant, fruit, and flower associated lactobacilli became exposed to animals that were feeding on their primary habitats. Although this exposure was initially coincidental, it is conceivable that bacterial traits that allowed the bacteria to tolerate the conditions in the host and allowed temporal persistence contributed to the transmission and hence, dispersal of lactobacilli. Given such traits would ultimately increase the success of the lineages in their primary habitats, they could be shaped by natural selection even if they did not allow stable colonization of the host, gradually increasing the relevance of host niches for the overall lifestyle. This would result in the evolution of distinct and dynamic lifestyles that differ in the degree by which the microbes rely on environmental and host niches and their dynamic interactions. Such 'nomadic' lifestyles remain represented within the genus

Lactobacillus and might well constitute a transitional state from the free-living lifestyle to a specialized symbiosis.

Consequences of lifestyle differences for host-microbe interrelationships

In symbiotic associations, both host and symbiont can reciprocally affect each other's evolution (Moran 2006). When stably associated with a host, the bacterial symbiont is likely to adapt, which can lead to specialization and host-restriction. The ultimate result is an obligate symbiosis, in which the microbe depends on the host for survival. This classic evolutionary process is represented within the lactobacilli. Lineages of *L. reuteri* have specialized to a degree that restricts their host range and although they are not yet obligate symbionts, this evolutionary process is likely to confine the ecology and evolutionary fate of lineages to particular host species (Frese *et al.* 2011 and 2013; Walter, Britton and Roos 2011). *L. iners* further progressed on the path to becoming an obligate symbiont, reducing its genome size to just over 1 Mb, resulting in fastidious nutritional requirements that surpass that of all other lactobacilli (Macklaim *et al.* 2011; Petrova *et al.* 2016). Several lineages of insect lactobacilli also evolved extensive levels of dependency on the host that resulted in a highly obligate lifestyle (Kwong and Moran 2016).

The consequences of this symbiosis for the host are less clear, mainly because we lack empirical data on the evolutionary outcomes in terms of measurable benefits for host fitness. However, some findings support the role of symbiotic lactobacilli in host fitness. Enzymes and metabolites produced by *Lactobacillus* species that colonize the proximal gastrointestinal tract of animals can aid in the digestive process and improve mineral availability (Gärtner 2002; McCabe *et al.* 2013). Lactate and other organic acids produced from fermentation prevent pathogens, e.g. *Enterobacteriaceae,* from growing in the chicken crop (Fuller 1974; Jin *et al.* 1996; Neal-McKinney *et al*. 2012), improve the efficiency of feed utilization, prevent diarrhea and regulate the immune system in pigs (Hou *et al.* 2015). Glycerol metabolism by human-lineage *L. reuteri* may contribute to the detoxification of heterocyclic amines (Engels *et al.* 2016). Data inferred from the genomes of *L. helsinborgensis* and related species indicates that they are able to metabolize a number of sugars that are indigestible and potentially toxic to bees, including mannose, galactose, lactose, arabinose and raffinose (Ellegaard *et al.* 2015). In the vagina, lactobacilli fulfill a protective role in the prevention of bacterial vaginosis (Borges, Silva and Teixeira 2014). Many host sites where lactobacilli exert a putative protective role have the characteristic of being lined by a stratified squamous tissue that allows *Lactobacillus* to adhere and form biofilms (Fig. 4). These epithelia might therefore constitute an anatomical feature that evolved in the host to facilitate specific colonization of the beneficial symbionts.

It remains unclear whether host-specificity in the *Lactobacillus*-host symbiosis enhances the beneficial outcomes for the host. Mutualism is often increased in specialized symbioses that are stably maintained over evolutionary times (Herre *et al.* 1999). Accordingly, the host-adapted species *L. reuteri* plays an important role in the development of immunological and neurological functions of the host (Zelante *et al.* 2013; Buffington *et al.* 2016; Lamas *et al.* 2016; He *et al.* 2017). However, the nomadic species *L. plantarum* also promotes growth of *Drosophila* (Storelli *et al.* 2011) even though it does not share a stable evolutionary history with this host. The common habitats (and food sources) of *Drosophila* and *L. plantarum* might be sufficient to align fitness interests and to establish a mutualistic association. The presence of *Drosophila* larvae seems to promote the long-term persistence of *L. plantarum* (Leulier *et al.* unpublished observations). Further research is required to systematically compare host-adapted strains with strains that do not share a natural history with particular hosts. In addition, adaptive evolution experiments similar to those performed to decipher the adaptation of a nomadic species to a specific host (van Bokhorst-van de Veen *et al.* 2013). could be expanded over extended experimental adaptation periods to also study the consequences of the evolutionary process on the fitness of the host. Such experiments can be performed in *Drosophila* (Ma *et al.* 2012).

Clearly, knowledge on the role of natural history on the beneficial effects of *Lactobacillus* species can inform research on the biological relevance of host-adapted species.

IMPLICATIONS OF AN UNDERSTANDING OF LIFESTYLES OF LACTOBACILLI

Bacterial taxonomy

Although a widely accepted molecular marker for the delineation of bacterial genera does not exist (Konstantinidis, Ramette and Tiedje 2006; Qin *et al.* 2014; Yarza *et al.* 2014) the diversity of the genus *Lactobacillus* far exceeds the diversity of typical bacterial families (Sun *et al.* 2015; Zheng *et al.* 2015a) while the phylogenetic groups within the *Lactobacillus sensu lato* (Fig. 2) represent taxonomic entities that are roughly equivalent to typical bacterial genera (Konstantinidis, Ramette and Tiedje 2006; Pot *et al.* 2014).These findings have led to proposals for a reclassification of the genus (Claesson, van Sinderen and O’Toole 2008). However, although clearly justified based on taxonomic considerations, the widespread use and recognition of the term “*Lactobacillus”* by the food and health-related industries, by laypersons, and in national or international regulations prevent adapting the taxonomy of the genus *Lactobacillus* to current scientific standards. Maintenance of genus name *Lactobacillus* in its current meaning conforms to the taxonomy of pathogenic or toxinogenic bacteria where species names are maintained even if they do not conform to current taxonomy, e.g. *Shigella* spp. or *Clostridium botulinum* (Collins and East 1998; Konstantinidis, Ramette and Tiedje 2006). Maintenance of the current genus name necessitates, however, the recognition of the phylogenetic groups in the genus *Lactobacillus sensu lato* as entities with distinct function and physiology, and, as outlined in this review, ecology and natural history. This recognition has many implications for the basic understanding of the biology of this group of organisms, as well as their important applications.

Knowledge about the real ecological niche of lactobacilli

Food environments are populated with free-living, nomadic or host-adapted organisms (Fig.1a), but as discussed above, the natural history of strains is for the most part unknown. The phylogenomic and evolutionary framework established here can aid in the identification of the lifestyle and real environmental niches of lactobacilli used in food and biotechnological applications. This can be combined with the analysis of metagenome datasets to determine the occurrence of species in different habitats (Lagkouvardos *et al.* 2016). We have verified this approach for a number of representative species found in food.

The analysis strongly supports a free-living lifestyle for the food-fermenting *L. parakefiri*. Phylogenetically, the species clusters with other environmental and plant isolates in the *L buchneri* group, with sequences corresponding to the species are mainly found in metagenomic datasets obtained from plants, animals that consume plants (rodents, cattle), and the human mouth (Fig. S1). In contrast, the framework clearly establishes the species *L. reuteri, L. salivarius* or *L. amylovorus,* which are also highly relevant in food fermentations (Vogel *et al.* 1999; Messens *et al.* 2002; Zheng *et al.* 2015b), as members of intestinal microbiota of humans or animals, which confirms previous findings on *L. reuteri* (Su *et al.* 2012). Interestingly, our analysis proposes the vertebrate gut as the real habitat of *L. helveticus* (Fig. 2 and Fig.S1) although the species is well recognized for its role in cheese production. Accordingly, several recent studies show that *L. helveticus* is a dominant member of chicken microbiota (Thitaram, Siragusa and Hinton 2005; Hammons *et al.* 2010; Robyn *et al.* 2012). The lifestyle of the food-fermenting *L. sanfranciscensis* remains more enigmatic. The species clusters in the insect-associated *L. fructivorans* group (Fig. 2), but the extremely infrequent detection of sequences representing *L. sanfranciscensis* and the related *L. vespulae* and *L. ixorae* in metagenome datasets from insects and marine invertebrates (Fig. S1) indicates that these strains are adapted to a very specific niche associated with invertebrates. Species in the *L. sakei* group are widely distributed in the environment and *L. sakei* occurs globally in fermented or spoiled meats, in silage, and in cereal fermentations (Chaillou *et al.* 2013; Fig. S1). Their psychrotrophic growth suggests an environmental origin in temperate or cold climates (Sakala *et al.* 2002; Chaillou *et al.* 2013) and they are found to be widely distributed in both host and plant-associated niches (Fig. S1), but their habitat remains unknown. Likewise, the origin of pediococci remains enigmatic. Their widespread distribution in food of plant and animal origin suggests a free-living or nomadic lifestyle, however, several *Pediococcus* spp. have a small genome size that may reflect niche specialization.

The inoculation or contamination of food and feed with fermentation or spoilage organisms is dependent on dispersal (Su *et al.* 2012; Andreevskaya *et al.* 2016; Broadbent *et al.* 2016; Gänzle and Ripari 2016). Knowledge about the real ecological origin of the lactobacilli therefore provides a powerful tool for the management and control of food fermentations and spoilage by taking appropriate measure to increase or decrease hurdles for dispersal of lactobacilli, respectively (Andreevskaya *et al.* 2016; Ripari, Gänzle and Berardi 2016). For this to be successful, future research should be focused on the elucidation of the natural history of lactobacilli found in food, particularly including understudied habitats that are less closely associated with humans and animals.

To inform the therapeutic use of *Lactobacillus* species

The list of *Lactobacillus* strains with recognized probiotic properties comprises host-adapted species such as *L. reuteri*, *L. johnsonii* and *L. acidophilus*, but also includes nomadic and free-living organisms such as *L. plantarum, L. casei* and *L. fermentum* (Floch *et al.* 2015). Moreover, sequences representing free-living and nomadic lactobacilli are as prevalent in intestinal metagenome datasets as those that are considered host-adapted (Fig. S1), supporting findings from previous human studies that commonly recovered these species by culture (Tannock *et al.* 2000; Walter *et al.* 2001; Dal Bello *et al.* 2003). This indicates that allochthonous lactobacilli are regularly introduced into the human gut through food and are frequently present and viable. It can therefore be concluded that lactobacilli, irrespective of lifestyle, can remain physiologically active in the gastrointestinal tract and have the potential to influence host physiology. However, the evolutionary history of an organism will determine how the bacteria interact with the host and is thus relevant for the selection of lactobacilli for therapeutic applications.

Host-adapted strains of lactobacilli show a higher ecological fitness in their respective hosts (Oh *et al.* 2010; Frese *et al.* 2011; Duar *et al.* 2017). Therefore, host-specific lactobacilli are more competitive when administered as a probiotic when compared to strains that do not share an evolutionary history with the host. Higher fitness is relevant for the development of probiotics aimed to outcompete pathogens and it is likely to be associated with higher metabolic activity in the host niche, which could lead to an increased production of metabolic compounds that define probiotic activity. In addition, stable transmission of bacterial symbionts over evolutionary times promotes traits that enhance partner performance (Herre *et al.* 1999; Sachs *et al.* 2004; Douglas 2008). Providing this theory holds true for the relationship between lactobacilli and animal hosts, then host-adapted *Lactobacillus* strains that share an evolutionary fate with their host are more likely to possess adaptive traits that enhance health of their host.

Such evolutionary aspects have rarely been considered for the selection of strains for specific applications. It is a logical working hypothesis that host-adapted *Lactobacillus* strains will show higher levels of ecological performance when used as probiotics, possess beneficial traits that enhance host fitness, and are likely to establish interactions with the host immune system that are characterized by tolerance (Walter, Britton and Roos 2011). Conversely, if the aim is to stimulate the immune system, selection of species or strains that lack a joint evolution with a host may be a more sensible approach. This proved true for *L. reuteri* in which strains from the autochthonous human lineage had an anti-inflammatory effect in human myeloid cells while strains associated with the phylogenetic lineage that evolved with poultry had a rather stimulatory immune effect (Spinler *et al.* 2014). These findings highlight the functional significance of the natural history of lactobacilli for probiotic functions. Although one cannot generalize what constitutes a better probiotic - host-adapted or not – the evolutionary history of a strain will fundamentally influence its functionality, and its consideration will therefore aid in the more systematic and targeted selection of optimal strains for specific therapeutic applications.

CONCLUDING REMARKS

More than a century after the genus *Lactobacillus* was first described (Beijerink, 1901; Orla-Jensen 1919), research continues to recover novel *Lactobacillus* species from habitats that have been hitherto uncharacterized. Moreover, advances in sequencing technologies increasingly support analysis of lactobacilli by large-scale comparative genomics. In addition to studies aiming to improve use of lactobacilli in fermented foods and health applications, the genomic information also allows elucidation of the adaptation of lactobacilli to diverse habitats and lifestyles We employed the available genomic information to develop a phylogenomic framework that allowed inferences regarding the natural history and lifestyle of lactobacilli and propose a model for the evolution of distinct lifestyles within the genus, which range from free-living to strictly host-adapted and symbiotic species. This model is well-supported by complementary approaches that include genomic, phylogenetic, ecological, and metabolic data and it provides a basis for the development of hypotheses on the evolutionary transitions that occurred within the genus. *Lactobacillus* species that do not share the lifestyle with other species in the same phylogenetic group are particularly relevant as models for the study of lifestyle transitions. Well-studied examples include *L. delbrueckii* ssp. *bulgaricus* and the *L. helsingorbensis* clade in the *L. delbrueckii* group, but this principle likely also applies for species such as *L. fermentum* and *L. senioris* (Fig. 2).

Although the evolutionary model that we propose is consistent with the available data, current knowledge has significant gaps with respect to the natural history of the genus. The currently available data on the occurrence of lactobacilli in the environment, or in diverse hosts is anthropocentric, as exemplified by the focus on food and domesticated vertebrates and insects as hosts for lactobacilli (Fig. 1 and 2). For many phylogenetic groups or species, we lack sufficient information to infer their real niche preference (Figure 2). Examples include host adapted species such as *L. senioris* and *L. sanfranciscensis*, free-living organisms in the *L. sakei* and *L. vaccinostercus* groups that show characteristics of adaptation to yet uncharacterized environmental niches, as well as organisms in the *L. alimentarius* group that may share the nomadic lifestyle of *L. plantarum* and *L. casei* but are much less well-studied. Even for well-characterized host-adapted species, we often know few details about how they maintain stable populations within host animals. Many species in the *L. delbrueckii* group including *L. gasseri*, *L. crispatus* and *L. acidophilus* are found in various vertebrate hosts and body sites (oral cavity, vagina, gastrointestinal tract), but we do not know whether they migrate among these niches and hosts, or whether specialized sub-populations within these species exist. We further know little about the role of lactobacilli in the oral cavity, although they are found in consistent high numbers, and could provide an essential niche and a source from which fecal isolates originate (Walter 2008). Future research to extend our knowledge on the natural history of *Lactobacillus* species will benefit from the phylogenomic framework established here and guided efforts that use large scale population genetic and comparative genomic approaches with strains or species found in different niches.

The implications of phylogenomic framework established here extend beyond its contribution to a basic understanding of the biology and ecology of the genus *Lactobacillus*. Humans have essentially ‘domesticated’ lactobacilli for use in food and feed production, and an increased understanding of the origin of these microbes and their function in nature will therefore facilitate the selection of strains for such applications. Attributes that lactobacilli that evolved in their natural habitats, such as metabolic functions, antagonism towards other members of microbial communities, and their impact on host species, can be exploited once understood. In addition, an understanding of host-associated lactobacilli might allow the development of strategies to support their populations or beneficial metabolic activities through dietary intervention. For example, strong attention has been paid towards the unsolved decline in the population of honey bees (Goulson *et al.* 2015; Engel *et al*. 2016). Their paramount importance as pollinators of agricultural crops justifies efforts to understand and better manage their symbiotic interactions with microbes as a tool to preserve insect health (Engel *et al*. 2016). Similar considerations also apply to humans and farm animals, many of which maintain dominant population of lactobacilli among their microbiota (i.e. swine and poultry). The framework presented here lays a foundation of strategies to support *Lactobacillus* populations and select strains for a whole range of biotechnological and therapeutic applications.

GLOSSARY OF TERMS

Adaptation: Process by which an organism becomes more fitted to an environment as the result of natural selection.

Allochthonous: Originates from a place other than that in which it is found.

Autochthonous: A true resident, found where formed.

Dispersal: Movements of individuals from a source location to another location where establishment and reproduction may occur.

Free-living: Associated with plant material and/or environment without relying on an eukaryotic host.

Habitat: The natural environment in which an organism lives.

Host-adapted: Specialized towards living in association with eukaryotic hosts, with adaptive traits that facilitates persistence

Lactobacillus sensu lato: (From Latin: “in the broad sense”). Includes the lactobacilli and related pediococci.

Lifestyle: The way of life of a species which allows its population to persist in nature.

Natural history: An organism's ecological interactions in its natural habitat and how they evolved.

Niche (Hutchinsonian niche)**:** Environmental conditions and resources within which a species can maintain a viable population

Nomadic: Dynamic lifestyle that involves both environmental and host niches, with no signs of specialization.

Specialized: Restricted in the breadth of its ecological niches as a result of trade-offs during adaptation**.**

Symbiosis (From Greek: *sym* “with” and *biosis* “living”) Long-term associations between genetically distinct organisms

End of GLOSSARY OF TERMS

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REFERENCES

Ahrné S, Nobaek S, Jeppsson B *et al.* The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. *J Appl Microbiol* 1998;**85**:88–94.

Alcántara C, Zúñiga M. Proteomic and transcriptomic analysis of the response to bile stress of *Lactobacillus casei* BL23. *Microbiology* 2012;**158**:1206–18.

Anderson AC, Sanunu M, Schneider C *et al.* Rapid species-level identification of vaginal and oral lactobacilli using MALDI-TOF MS analysis and 16S rDNA sequencing. *BMC Microbiol* 2014;**14**:312.

Anderson KEK, Sheehan THTTH, Mott BMB *et al.* Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). *PLoS One* 2013;**8**:e83125.

Andreevskaya M. Ecological fitness and interspecies interactions of food-spoilage-associated lactic acid bacteria: insights from the genome analyses and transcriptome profiles. Dissertation, University of Helsinki 2017. urn.fi/URN:ISBN:978-951-51-2832-4

Andreevskaya M, Johansson P, Jääskeläinen E *et al. Lactobacillus oligofermentans* glucose, ribose and xylose transcriptomes show higher similarity between glucose and xylose catabolism-induced responses in the early exponential growth phase. *BMC Genomics* 2016;**17**:539.

Battistuzzi FU, Feijao A, Hedges SB et al. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC Evol Biol*

2004;**4**:44.

Beijerink MW. Anhäufungsversuche mit Ureumbakterien: Ureumspaltung durch Urease und durch Katabolismus. Zentralbl Bakteriol Parasitenkde Infektionskrankh Hyg Abt 2 1901;**7**:33–61.

van Bokhorst-van de Veen H, Smelt MJ, Wels M *et al.* Genotypic adaptations associated with prolonged persistence of *Lactobacillus plantarum* in the murine digestive tract. *Biotechnol J* 2013;**8**:895–904.

Borges S, Silva J, Teixeira P. The role of lactobacilli and probiotics in maintaining vaginal health. *Arch Gynecol Obstet* 2014;**289**:479–89.

Bringel F, Curk MC, Hubert JC. Characterization of lactobacilli by southern-type hybridization with a *Lactobacillus plantarum pyrDFE* probe. *Int J Syst Bacteriol* 1996;**46**:588–94.

Broadbent JR, Neeno-Eckwall EC, Stahl B *et al.* Analysis of the *Lactobacillus casei* supragenome and its influence in species evolution and lifestyle adaptation. *BMC Genomics* 2012;**13**:533.

Broadbent JR, Oberg TS, Oberg CJ *et al. Lactobacillus wasatchensis* sp. nov., a non-starter lactic acid bacteria isolated from aged Cheddar cheese. *Int J Syst Evol Microbiol* 2016;**66**:158–64.

Bron PA., van Baarlen P, Kleerebezem M. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nat Rev Microbiol* 2011;**10**:66–78.

Bron PA, Grangette C, Mercenier A *et al.* Identification of *Lactobacillus plantarum* genes that are induced in the gastrointestinal tract of mice. *J Bacteriol* 2004a;**186**:5721–9.

Bron PA, Marco M, Hoffer SM *et al.* Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters *in vitro* and *in situ* in the gastrointestinal tract. *J Bacteriol* 2004b;**186**:7829–35.

Brooijmans RJW, de Vos WM, Hugenholtz J. *Lactobacillus plantarum* wcfs1 electron transport chains. *Appl Environ Microbiol* 2009;**75**:3580–5.

Buffington SA, Di Prisco GV, Auchtung TA *et al.* Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* 2016;**165**:1762–75.

Buhnik-Rosenblau K, Matsko-Efimov V, Jung M *et al.* Indication for co-evolution of *Lactobacillus johnsonii* with its hosts. *BMC Microbiol* 2012;**12**:149.

Cai H, Rodríguez BT, Zhang W *et al.* Genotypic and phenotypic characterization of *Lactobacillus casei* strains isolated from different ecological niches suggests frequent recombination and niche specificity. *Microbiology* 2007;**153**:2655–65.

Cai H, Thompson R, Budinich MF *et al.* Genome sequence and comparative genome analysis of *Lactobacillus casei*: insights into their niche-associated evolution. *Genome Biol Evol* 2009;**1**:239–57.

Ceapa C, Davids M, Ritari J *et al*. The variable regions of *lactobacillus rhamnosus* genomes reveal the dynamic evolution of metabolic and host-adaptation repertoires. *Genome Biol Evol* 2016;**8**:1889–905.

Chaillou S, Champomier-Vergès M-C, Cornet M et al. The complete genome sequence of the meat-borne lactic acid bacterium *Lactobacillus sakei* 23K. *Nat Biotechnol* 2005;**23**:1527–33.

Chaillou S, Lucquin I, Najjari A *et al.* Population genetics of *Lactobacillus sakei* reveals three lineages with distinct evolutionary histories. *PLoS One* 2013;**8**:e73253.

Chandler JA, Lang J, Bhatnagar S *et al.* Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLoS Genet* 2011;**7**:e1002272.

Chang Y-C, Tsai C-Y, Lin C-F et al. Characterization of tetracycline resistance lactobacilli isolated from swine intestines at western area of Taiwan. *Anaerobe* 2011;**17**:239–45.

Claesson MJ, van Sinderen D, O'Toole PW. *Lactobacillus* phylogenomics - towards a reclassification of the genus. *Int J Syst Evol Microbiol* 2008;**58**:2945–54.

Clarke JT, Warnock RCM, Donoghue PCJ. Establishing a time-scale for plant evolution. *New Phytol* 2011;**192**:266–301.

Collins MD, East AK. Phylogeny and taxonomy of the food-borne pathogen *Clostridium botulinum* and its neurotoxins. *J Appl Microbiol* 1998;**84**:5–17.

Daeschel MAA, Andersson REE, Fleming HPP. Microbial ecology of fermenting plant materials. *FEMS Microbiol Lett* 1987;**46**:357–67.

Dal Bello F, Hertel C. Oral cavity as natural reservoir for intestinal lactobacilli. *Syst Appl Microbiol* 2006;**29**:69–76.

Dal Bello F, Walter J, Hammes WP *et al.* Increased complexity of the species composition of lactic acid bacteria in human feces revealed by alternative incubation condition. *Microb Ecol* 2003;**45**:455–63.

Danner H, Holzer M, Mayrhuber E *et al.* Acetic acid increases stability of silage under aerobic conditions. *Appl Environ Microbiol* 2003;**69**:562–7.

Delgado S, Suárez A, Mayo B. Dominant cultivable *Lactobacillus* species from the feces of healthy adults in northern Spain. *Int Microbiol* 2010;**10**:141–5.

Dewar WA, McDonald P, Whittenbury R. The hydrolysis of grass hemicelluloses during ensilage. *J Sci Food Agric* 1963;**14**:411–7.

Diancourt L, Passet V, Chervaux C *et al.* Multilocus sequence typing of *Lactobacillus casei* reveals a clonal population structure with low levels of homologous recombination. *Appl*

Environ Microbiol 2007;**73**:6601–11.

Dieckmann U, O’Hara B, Weisser W. The evolutionary ecology of dispersal. *Trends Ecol Evol* 1999;**14**:88–90.

Ding XF, Wu C De, Zhang LQ et al. Characterization of eubacterial and archaeal community diversity in the pit mud of Chinese Luzhou-flavor liquor by nested PCR-DGGE. *World J Microbiol Biotechnol* 2014;**30**:605–12.

Douglas AE. Conflict, cheats and the persistence of symbioses. *New Phytol* 2008;**177**:849–58.

Douillard FP, Ribbera A, Järvinen HM *et al.* Comparative genomic and functional analysis of lactobacillus casei and *Lactobacillus rhamnosus* strains marketed as probiotics. *Appl Environ Microbiol* 2013;**79**:1923–33.

Duar RM, Frese SA, Fernando SC *et al.* Experimental determination of host adaptation of *Lactobacillus reuteri* to different vertebrate species. *Appl Environ Microbiol* 2017, *in press,* doi: 10.1128/AEM.00132-17.

Ellegaard KM, Tamarit D, Javelind E *et al.* Extensive intra-phylotype diversity in lactobacilli and bifidobacteria from the honeybee gut. *BMC Genomics* 2015;**16**:284.

Endo A, Futagawa-Endo Y, Dicks LMT. Isolation and characterization of fructophilic lactic acid bacteria from fructose-rich niches. *Syst Appl Microbiol* 2009;**32**:593–600.

Endo A, Futagawa-Endo Y, Sakamoto M *et al. Lactobacillus florum* sp. nov., a fructophilic species isolated from flowers. *Int J Syst Evol Microbiol* 2010;**60**:2478–82.

Endo A, Irisawa T, Futagawa-Endo Y *et al.* Characterization and emended description of *Lactobacillus kunkeei* as a fructophilic lactic acid bacterium. *Int J Syst Evol Microbiol* 2012;**62**:500–4.

Engel P, Moran NA. The gut microbiota of insects – diversity in structure and function. *FEMS*

Microbiol Rev 2013;**37**:699–735.

Engel P, Kwong WK, McFrederick Q *et al.* The bee microbiome: impact on bee health and model for evolution and ecology of host-microbe interactions. *MBio* 2016;**7**:e02164-15.

Engels C, Schwab C, Zhang J *et al.* Acrolein contributes strongly to antimicrobial and heterocyclic amine transformation activities of reuterin. *Sci Rep* 2016;**6**:36246.

Erkosar B, Storelli G, Defaye A *et al.* Host-intestinal microbiota mutualism: “learning on the fly.” *Cell Host Microbe* 2013;**13**:8–14.

Fenton MP. An investigation into the sources of lactic acid bacteria in grass silage. *J Appl Bacteriol* 1987;**62**:181–8.

Filannino P, Di Cagno R, Addante R *et al.* Metabolism of fructophilic lactic acid bacteria isolated from *Apis mellifera* L. bee-gut: a focus on the phenolic acids as external electron acceptors. *Appl Environ Microbiol* 2016;**82**: 6899-911.

Floch MH, Walker WA, Sanders ME *et al.* Recommendations for Probiotic Use--2015 Update: Proceedings and Consensus Opinion. *J Clin Gastroenterol* 2015;**49 Suppl 1**:S69-73.

Frese SA, Benson AK, Tannock GW *et al.* The evolution of host specialization in the vertebrate gut symbiont *Lactobacillus reuteri*. *PLoS Genet* 2011;**7**:16.

Frese SA, Mackenzie DA, Peterson DA *et al.* Molecular characterization of host-specific biofilm formation in a vertebrate gut symbiont. *PLoS Genet* 2013;**9**:e1004057.

Fuller R, Brooker BE. Lactobacilli which attach to the crop epithelium of the fowl. *Am J Clin Nutr* 1974;**27**:1305–12.

Galdeano CM, Perdigón G. Role of viability of probiotic strains in their persistence in the gut and in mucosal immune stimulation. *J Appl Microbiol* 2004;**97**:673–81.

Gänzle M, Ripari V. Composition and function of sourdough microbiota: From ecological theory to bread quality. *Int J Food Microbiol* 2016;**239**:19–25.

Gänzle MG. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. *Curr Opin Food Sci* 2015;**2**:106–17.

Gänzle MG, Follador R. Metabolism of oligosaccharides and starch in lactobacilli: a review. *Front Microbiol* 2012;**3**:340.

Gänzle MG, Vermeulen N, Vogel RF. Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiol* 2007;**24**:128–38.

Gärtner K. The forestomach of rats and mice, an effective device supporting digestive metabolism in muridae (review). *J Exp Anim Sci* 2002;**42**:1–20

Goulson D, Nicholls E, Botias C et al. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 2015;**347**:1255957–1255957.

Grill JP, Cayuela C, Antoine JM *et al.* Isolation and characterization of a *Lactobacillus amylovorus* mutant depleted in conjugated bile salt hydrolase activity: relation between activity and bile salt resistance. *J Appl Microbiol* 2000;**89**:553–63.

Guan LL, Hagen KE, Tannock GW et al. Detection and identification of *Lactobacillus* species in crops of broilers of different ages by using PCR-denaturing gradient gel electrophoresis and amplified ribosomal DNA restriction analysis. *Appl Environ Microbiol* 2003;**69**:6750–7.

van de Guchte M, Penaud S, Grimaldi C *et al.* The complete genome sequence *of Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. *Proc Natl Acad Sci U S A* 2006;**103**:9274–9.

Hallmann J, Quadt-Hallmann A, Mahaffee WF *et al.* Bacterial endophytes in agricultural crops. *Can J Microbiol* 1997;**43**:895–914.

Hammes WP, Hertel C. The genera *Lactobacillus* and *Carnobacterium*. *The Prokaryotes*. New

York, NY, NY: Springer US, 2006, 320–403.

Hammes WP, Vogel RF. The genus *Lactobacillus*. *The Genera of Lactic Acid Bacteria*. Boston, MA: Springer US, 1995, 19–54.

Hammons S, Oh PL, Martínez I *et al.* A small variation in diet influences the *Lactobacillus* strain composition in the crop of broiler chickens. *Syst Appl Microbiol* 2010;**33**:275–81.

Hayden B, Canuel N, Shanse J. What was brewing in the natufian? An archaeological assessment of brewing technology in the epipaleolithic. *J Archaeol Method Theory* 2013;**20**:102–50.

He B, Hoang TK, Wang T *et al.* Resetting microbiota by *Lactobacillus reuteri* inhibits T reg deficiency–induced autoimmunity via adenosine A2A receptors. *J Exp Med* 2017;**214**:107-23.

Hedges SB, Blair JE, Venturi ML *et al.* A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evol Biol* 2004;**4**:2.

Heilig HGHJ, Zoetendal EG, Vaughan EE *et al.* Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Appl Environ Microbiol* 2002;**68**:114–23.

Heinl S, Wibberg D, Eikmeyer F *et al.* Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. *J Biotechnol* 2012;**161**:153–66

Herre E, Knowlton N, Mueller U *et al.* The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol Evol* 1999;**14**:49–53.

Hill C, Guarner F, Reid G *et al.* Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014;**11**:506-14.

Hou C, Zeng X, Yang F *et al.* Study and use of the probiotic *Lactobacillus reuteri* in pigs: a review. *J Anim Sci Biotechnol* 2015;**6**:14.

Jacobs MJ, Bugbee WM, Gabrielson DA. Enumeration, location, and characterization of endophytic bacteria within sugar beet roots. *Can J Bot* 1985;**63**:1262–5.

Jin LZ, Ho YW, Abdullah N et al. Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. *Lett Appl Microbiol* 1996;**23**:67–71.

Kakimoto S, Okazaki K, Sakane T *et al.* Isolation and taxonomic characterization of acid urease-producing bacteria. *Agric Biol Chem* 1989;**53**:1111–8.

Kandler O, Weiss N. Genus *Lactobacillus*. In: Sneath P, Mair N, Sharpe M, et al. (eds.). *Bergey's Manual of Systematic Bacteriology*. 9th ed. Williams & Wilkins, 1986, 1063–5.

Kankainen M, Paulin L, Tynkkynen S *et al.* Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proc Natl Acad Sci U S A* 2009;**106**:17193–8.

Kitara K, Kaneko T, Goto O. Taxonomic studies on the hiochi-bacteria, specific saprophytes of sake. II. Identification and classification of hiochi-bacteria. *J Gen Appl Microbiol* 1957;**3**:111–20.

Klein G, Pack A, Bonaparte C et al. Taxonomy and physiology of probiotic lactic acid bacteria. *Int J Food Microbiol* 1998;**41**:103–25.

Konstantinidis KT, Ramette A, Tiedje JM. The bacterial species definition in the genomic era. *Philos Trans R Soc Lond B Biol Sci* 2006;**361**:1929–40.

Konstantinov SR, Awati AA, Williams BA et al. Post-natal development of the porcine microbiota composition and activities. *Environ Microbiol* 2006;**8**:1191–9.

Konstantinov SR, Awati A, Smidt H et al. Specific response of a novel and abundant *Lactobacillus amylovorus*-like phylotype to dietary prebiotics in the guts of weaning piglets. *Appl Environ Microbiol* 2004;**70**:3821–30.

Krumbeck JA, Marsteller NL, Frese SA *et al.* Characterization of the ecological role of genes mediating acid resistance in *Lactobacillus reuteri* during colonization of the gastrointestinal tract. *Environ Microbiol* 2016;**18**:2172–84.

Kvasnikov EI, Kovalenko NK, Nesterenko OA. Lactic acid bacteria in nature and the national economy. *Appl Biochem Microbiol* 1983;**18**:665–76.

Kwong WK, Moran NA. Gut microbial communities of social bees. *Nat Rev Microbiol* 2016;**14**:374–84.

Kwong WK, Moran NA. Evolution of host specialization in gut microbes: the bee gut as a model. *Gut Microbes* 2015;**6**:214–20.

Lagkouvardos I, Joseph D, Kapfhammer M *et al.* IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. *Sci Rep* 2016;**6**:33721.

Lähteinen T, Malinen E, Koort JMK *et al.* Probiotic properties of *Lactobacillus* isolates originating from porcine intestine and feces. *Anaerobe* 2010;**16**:293–300.

Lamas B, Richard ML, Leducq V *et al.* CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. *Nat Med* 2016;**22**:598–605.

De Las Rivas B, Marcobal ÁE, Gómez A *et al.* Characterization of ISLpl4, a functional insertion sequence in *Lactobacillus plantarum*. *Gene* 2005;**363**:202–10.

Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev* 2008;**72**:728–64.

Leser TD, Amenuvor JZ, Jensen TK et al. Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* 2002;6**8**:673–90.

Lo W-SS, Huang Y-YY, Kuo C-HH. Winding paths to simplicity: genome evolution in facultative insect symbionts. *FEMS Microbiol Rev* 2016;**40**:855–74.

Luo Z-X. Transformation and diversification in early mammal evolution. *Nature* 2007;**450**:1011–9.

Mackenzie DA, McLay K, Roos S *et al.* Draft genome sequence of a novel *Lactobacillus salivariu*s strain isolated from piglet. *Genome Announc* 2014;**2**: e01231-13.

Macklaim JM, Gloor GB, Anukam KC *et al.* At the crossroads of vaginal health and disease, the genome sequence of *Lactobacillus iners* AB-1. *Proc Natl Acad Sci U S A* 2011;**108 Suppl 1**:4688–95.

Maeno S, Tanizawa Y, Kanesaki Y *et al.* Genomic characterization of a fructophilic bee symbiont *Lactobacillus kunkeei* reveals its niche-specific adaptation. *Syst Appl Microbiol* 2016;**39**:516–26.

Ma J, Benson A, Kachman S *et al. Drosophila melanogaster* selection for survival of *Bacillus cereus* infection: life history trait indirect responses. *Int J Evol Biol* 2012; **2012**: 935970.

Makarova K, Slesarev A, Wolf Y et al. Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci U S A* 2006;**103**:15611–6.

Mann E, Schmitz-Esser S, Zebeli Q *et al.* Mucosa-associated bacterial microbiome of the gastrointestinal tract of weaned pigs and dynamics linked to dietary calcium-phosphorus. *PLoS One* 2014;**9**: e86950.

Marco ML, Pavan S, Kleerebezem M. Towards understanding molecular modes of probiotic action. *Curr Opin Biotechnol* 2006;**17**:204–10.

Martín R, Jiménez E, Olivares M *et al. Lactobacillus salivarius* CECT 5713, a potential probiotic strain isolated from infant feces and breast milk of a mother–child pair. *Int J Food Microbiol* 2006;**112**:35–43.

Martínez I, Stegen JC, Maldonado-Gómez MX *et al.* The gut microbiota of rural Papua New Guineans: composition, diversity patterns, and ecological processes. *Cell Rep* 2015;**11**:527–38.

Marti R, Dabert P, Ziebal C *et al.* Evaluation of *Lactobacillus sobrius/L. amylovorus* as a new microbial marker of pig manure. *Appl Environ Microbiol* 2010;**76**:1456–61.

Martino ME, Bayjanov JR, Caffrey BE *et al.* Nomadic lifestyle of *Lactobacillus plantarum* revealed by comparative genomics of 54 strains isolated from different habitats. *Environ Microbiol* 2016;**18**:4974–89.

Matos RC, Leulier F. Lactobacilli-host mutualism: “learning on the fly.” *Microb Cell Fact* 2014;**13**:S6.

McCabe LR, Irwin R, Schaefer L *et al*. Probiotic use decreases intestinal inflammation and increases bone density in healthy male but not female mice. *J Cell Physiol* 2013;**228**:1793–8.

McFrederick QS, Cannone JJ, Gutell RR *et al.* Specificity between lactobacilli and hymenopteran hosts is the exception rather than the rule. *Appl Environ Microbiol* 2013;**79**:1803–12.

McFrederick QS, Wcislo WT, Taylor DR *et al.* Environment or kin: whence do bees obtain acidophilic bacteria? *Mol Ecol* 2012;**21**:1754–68.

de Melo Pereira GV, Magalhães KT, Lorenzetii ER *et al.* A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. *Microb Ecol* 2012;**63**:405–17.

Mendes-Soares H, Suzuki H, Hickey RJ *et al.* Comparative functional genomics of *Lactobacillus* spp. reveals possible mechanisms for specialization of vaginal lactobacilli to their environment. *J Bacteriol* 2014;**196**:1458–70.

Mercier J, Lindow SE. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Appl Environ Microbiol* 2000;**66**:369–74.

Messens W, Neysens P, Vansieleghem W *et al.* Modeling growth and bacteriocin production by *Lactobacillus amylovorus* DCE 471 in response to temperature and pH values used for sourdough fermentations. *Appl Environ Microbiol* 2002;**68**:1431–5.

Minervini F, Celano G, Lattanzi A *et al.* Lactic acid bacteria in durum wheat flour are endophytic components of the plant during its entire life cycle. *Appl Environ Microbiol* 2015;**81**:6736–48.

Misof B, Liu S, Meusemann K *et al.* Phylogenomics resolves the timing and pattern of insect evolution. *Science* 2014;**346**: 763-7.

Molenaar D, Bringel F, Schuren FH *et al.* Exploring *Lactobacillus plantarum* genome diversity by using microarrays. *J Bacteriol* 2005;**187**:6119–27.

Molin G, Jeppsson B, Johansson M-L *et al.* Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. *J Appl Bacteriol* 1993;**74**:314–23.

Mora D, Arioli S, Compagno C. Food environments select microorganisms based on selfish energetic behavior. *Front Microbiol* 2013;**4**:348.

Moran NA. Symbiosis. *Curr Biol* 2006;**16**:R866–71.

Müller M, Lier D. Fermentation of fructans by epiphytic lactic acid bacteria. *J Appl Bacteriol*

1994;**76**:406–11.

Mundt JO, Hammer JL. Lactobacilli on plants. *Appl Microbiol* 1968;**16**:1326–30.

Nakamura LK. *Lactobacillus amylovorus*, a new starch-hydrolyzing species from cattle waste-corn fermentations. *Int J Syst Bacteriol* 1981;**31**:56–63.

Neal-McKinney JM, Lu X, Duong T et al. Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. *PLoS One* 2012;**7**:e43928.

Neveling DP, Endo A, Dicks LMT. Fructophilic *Lactobacillus kunkeei* and *Lactobacillus brevis* isolated from fresh flowers, bees and bee-hives. *Curr Microbiol* 2012;**65**:507–15.

van den Nieuwboer M, van Hemert S, Claassen E *et al. Lactobacillus Plantarum* WCFS1 and its host interaction: a dozen years after the genome. *Microb Biotechnol* 2016;**9**:452–65.

O' Donnell MM, Harris HMB, Lynch DB *et al. Lactobacillus ruminis* strains cluster according to their mammalian gut source. *BMC Microbiol* 2015;**15**:80.

Oh PL, Benson AK, Peterson DA *et al.* Diversification of the gut symbiont *Lactobacillus reuteri* as a result of host-driven evolution. *ISME J* 2010;**4**:377–87.

Orla-Jensen S. *The Lactic Acid Bacteria*. Copenhagen: Andr. Fred. Host & Son, 1919.

Pedersen K, Tannock GW. Colonization of the porcine gastrointestinal tract by lactobacilli. *Appl Envir Microbiol* 1989;**55**:279–83.

Pedersen MB, Gaudu P, Lechardeur D *et al.* Aerobic respiration metabolism in lactic acid bacteria and uses in biotechnology. *Annu Rev Food Sci Technol* 2012;**3**:37–58.

Petrova MI, Reid G, Vaneechoutte M *et al. Lactobacillus iners*: Friend or Foe? *Trends Microbiol* 2016;**xx**:1–10.

Pires ND, Dolan L. Morphological evolution in land plants: new designs with old genes. *Philos Trans R Soc B Biol Sci* 2012;**367**:508–18.

Pontonio E, Mahony J, Di Cagno R *et al.* Cloning, expression and characterization of a β-D-xylosidase from *Lactobacillus rossiae* DSM15814(T). *Microb Cell Fact* 2016;**15**:72.

Pot B, Felis GE, Bruyne K De *et al.* The genus *Lactobacillus*. *Lactic Acid Bacteria*. Chichester, UK: John Wiley & Sons, Ltd, 2014, 249–353.

Powell JE, Leonard SP, Kwong WK *et al.* Genome-wide screen identifies host colonization determinants in a bacterial gut symbiont. *Proc Natl Acad Sci U S A* 2016;**113**:13887–92.

Qin Q-L, Xie B-B, Zhang X-Y *et al.* A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 2014;**196**:2210–5.

Raftis EJ, Salvetti E, Torriani S *et al.* Genomic diversity of *Lactobacillus salivarius*. *Appl Environ Microbiol* 2011;**77**:954–65.

Regmi PR, Metzler-Zebeli BU, Ganzle MG *et al.* starch with high amylose content and low in vitro digestibility increases intestinal nutrient flow and microbial fermentation and selectively promotes bifidobacteria in pigs. *J Nutr* 2011;**141**:1273–80.

Reti KL, Thomas MC, Yanke LJ et al. Effect of antimicrobial growth promoter administration on the intestinal microbiota of beef cattle. Gut Pathog 2013;**5**:8.

Reuter G. The *Lactobacillus* and *Bifidobacterium* microflora of the human intestine: composition and succession. *Curr Issues Intest Microbiol* 2001;**2**:43–53.

Ricciardi A, Ianniello RG, Parente E *et al.* Modified chemically defined medium for enhanced respiratory growth of *Lactobacillus casei* and *Lactobacillus plantarum* groups. *J Appl Microbiol* 2015;**119**:776–85.

Ripari V, Gänzle MG, Berardi E. Evolution of sourdough microbiota in spontaneous sourdoughs started with different plant materials. *Int J Food Microbiol* 2016;**232**:35–42.

Robyn J, Rasschaert G, Messens W *et al.* Screening for lactic acid bacteria capable of inhibiting

Campylobacter jejuni in *in vitro* simulations of the broiler chicken caecal environment. *Benef Microbes* 2012;**3**:299–308.

Rogosa M, Wiseman RF, Mitchell JA *et al.* Species differentiation of oral lactobacilli from man including description of *Lactobacillus salivarius* nov spec and *Lactobacillus cellobiosus* nov spec. *J Bacteriol* 1953;**65**:681–99.

Rossi M, Martínez-Martínez D, Amaretti A *et al.* Mining metagenomic whole genome sequences revealed subdominant but constant *Lactobacillus* population in the human gut microbiota. *Environ Microbiol* 2016;**8**:399-406.

Ruiz L, Margolles A, Sánchez B. Bile resistance mechanisms in *Lactobacillus* and *Bifidobacterium*. *Front Microbiol* 2013;**4**:396.

Sachs J, Skophammer R, Regus J. Evolutioanry transitions in bacterial symbiosis. *Proc Natl Acad Sci U S A* 2011;**108**:10800–7.

Sachs JL, Mueller UG, Wilcox TP *et al.* The evolution of cooperation. *Q Rev Biol* 2004;**79**:135–60.

Sakala RM, Kato Y, Hayashidani H *et al. Lactobacillus fuchuensis* sp. nov., isolated from vacuum-packaged refrigerated beef. *Int J Syst Evol Microbiol* 2002;**52**:1151–4.

Salvetti E, Torriani S, Felis GE. The genus *Lactobacillus*: a taxonomic update. *Probiotics Antimicrob Proteins* 2012;**4**:217–26.

Savijoki K, Ingmer H, Varmanen P. Proteolytic systems of lactic acid bacteria. *Appl Microbiol Biotechnol* 2006;**71**:394–406.

Scheirlinck I, Van der Meulen R, De Vuyst L *et al.* Molecular source tracking of predominant lactic acid bacteria in traditional Belgian sourdoughs and their production environments. *J Appl Microbiol* 2009;**106**:1081–92.

Sharpe ME, Fryer TF, Smith DG. Identification of the lactic acid bacteria. In: Gibbs BM, Skinner F. (eds.). *Identification Methods for Microbiologists, Part A*. London, 1966, 65–79.

Shetty S, Griffin DK, Graves JAM. Comparative painting reveals strong chromosome homology over 80 million years of bird evolution. *Chromosom Res* 1999;**7**:289–95.

Shrivastava KPP. Bacterium (*Lactobacillus*?) eurydice strains from bumble bees. J Invertebr Pathol 1982;**40**:180–5.

Siezen RJ, van Hylckama Vlieg JET. Genomic diversity and versatility of *Lactobacillus plantarum*, a natural metabolic engineer. *Microb Cell Fact* 2011;**10 Suppl 1**:S3.

Siezen RJ, Tzeneva VA, Castioni A *et al.* Phenotypic and genomic diversity of *Lactobacillus plantarum* strains isolated from various environmental niches. *Environ Microbiol* 2010;**12**:758–73.

Smokvina T, Wels M, Polka J *et al. Lactobacillus paracasei* comparative genomics: towards species pan-genome definition and exploitation of diversity. *PLoS One* 2013;**8**:e68731.

Spinler JK, Sontakke A, Hollister EB *et al.* From prediction to function using evolutionary genomics: human-specific ecotypes of *Lactobacillus reuteri* have diverse probiotic functions. *Genome Biol Evol* 2014;**6**:1772–89.

Spor A, Nidelet T, Simon J *et al.* Niche-driven evolution of metabolic and life-history strategies in natural and domesticated populations of *Saccharomyces cerevisiae*. *BMC Evol Biol* 2009;**9**:296.

Stefanovic E, Fitzgerald G, McAuliffe O. Advances in the genomics and metabolomics of dairy lactobacilli: A review. *Food Microbiol* 2017;**61**:33–49.

Steinkraus KH. Fermentations in world food processing. *Compr Rev Food Sci Food Saf* 2002;**1**:23–32.

Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol* 1997;**36**:1–29.

Stirling AC, Whittenbury R. Sources of the lactic acid bacteria occurring in silage. *J Appl Bacteriol* 1963;**26**:86–90.

Storelli G, Defaye A, Erkosar B *et al.* *Lactobacillus plantarum* promotes *Drosophila* systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab* 2011;**14**:403–14.

Su MS-W, Oh PL, Walter J *et al.* Intestinal origin of sourdough *Lactobacillus reuteri* isolates as revealed by phylogenetic, genetic, and physiological analysis. *Appl Environ Microbiol* 2012;**78**:6777–80.

Sun Z, Harris HMB, McCann A *et al.* Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nat Commun* 2015;**6**:8322.

Tailliez P. Mini-revue : les bactéries lactiques,ces êtres vivants apparus il y a près de 3 milliards d’années. *Lait* 2001;**81**:1–11.

Tamarit D, Ellegaard KM, Wikander J *et al.* Functionally structured genomes in *Lactobacillus kunkeei* colonizing the honey crop and food products of honeybees and stingless bees. *Genome Biol Evol* 2015;**7**:1455–73.

Tanizawa Y, Tohno M, Kaminuma E *et al.* Complete genome sequence and analysis of *Lactobacillus hokkaidonensis* LOOC260T, a psychrotrophic lactic acid bacterium isolated from silage. *BMC Genomics* 2015;**16**:240.

Tannock GW. The lactic microflora of pigs, mice and rats. *The Lactic Acid Bacteria Volume 1*. Boston, MA, MA: Springer US, 1992, 21–48.

Tannock GW. A special fondness for lactobacilli. *Appl Environ Microbiol* 2004;**70**:3189–94.

Tannock GW, Munro K, Harmsen HJ *et al.* Analysis of the fecal microflora of human subjects consuming a probiotic product containing *Lactobacillus rhamnosus* DR20. *Appl Environ Microbiol* 2000;**66**:2578–88.

Tannock GW, Wilson CM, Loach D *et al.* Resource partitioning in relation to cohabitation of *Lactobacillus* species in the mouse forestomach. *ISME J* 2012;**6**:927–38.

Techo S, Miyashita M, Shibata C *et al. Lactobacillus ixorae* sp. nov., isolated from a flower (West-Indian jasmine). *Int J Syst Evol Microbiol* 2016;**66**:5500–5.

Thitaram SN, Siragusa GR, Hinton A. *Bifidobacterium*-selective isolation and enumeration from chicken caeca by a modified oligosaccharide antibiotic-selective agar medium. *Lett Appl Microbiol* 2005;**41**:355–60.

Tohno M, Kitahara M, Uegaki R et al. *Lactobacillus hokkaidonensis* sp. nov., isolated from subarctic timothy grass (*Phleum pratense L.*) silage. *Int J Syst Evol Microbiol* 2013;**63**:2526–31.

Torriani S, Clementi F, Vancanneyt M *et al.* Differentiation of *Lactobacillus plantarum*, *L. pentosus* and *L. paraplantarum* species by RAPD-PCR and AFLP. *Syst Appl Microbiol* 2001;**24**:554–60.

Vásquez A, Forsgren E, Fries I *et al.* Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One* 2012;**7**:e33188.

Vellend M. Conceptual synthesis in community ecology. *Q Rev Biol* 2010;**85**:183–206.

Vera Pingitore E, Hébert EM, Nader-Macías ME *et al.* Characterization of salivaricin CRL 1328, a two-peptide bacteriocin produced by *Lactobacillus salivarius* CRL 1328 isolated from the human vagina. *Res Microbiol* 2009;**160**:401–8.

Vogel RF, Knorr R, Müller MR *et al.* Non-dairy lactic fermentations: the cereal world. *Antonie Van Leeuwenhoek* 1999;**76**:403–11.

Vogel RF, Pavlovic M, Ehrmann MA *et al.* Genomic analysis reveals *Lactobacillus sanfranciscensis* as stable element in traditional sourdoughs. *Microb Cell Fact* 2011;**10 Suppl 1**:S6.

Vojvodic S, Rehan SM, Anderson KKE *et al.* Microbial gut diversity of Africanized and European honey bee larval instars. *PLoS One* 2013;**8**:e72106.

Wall R, Fitzgerald G, Hussey S *et al.* Genomic diversity of cultivable *Lactobacillus* populations residing in the neonatal and adult gastrointestinal tract. *FEMS Microbiol Ecol* 2007;**59**:127–37.

Walter J. Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol* 2008;**74**:4985–96.

Walter J, Britton RA, Roos S. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the *Lactobacillus reuteri* paradigm. *Proc Natl Acad Sci U S A* 2011;**108 Suppl**:4645–52.

Walter J, Chagnaud P, Tannock GW *et al.* A high-molecular-mass surface protein (Lsp) and methionine sulfoxide reductase B (MsrB) contribute to the ecological performance of *Lactobacillus reuteri* in the murine gut. *Appl Environ Microbiol* 2005;**71**:979–86.

Walter J, Hertel C, Tannock GW *et al.* Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR Primers and Denaturing Gradient Gel Electrophoresis. *Appl Environ Microbiol* 2001;**67**:2578–85.

Walter J, Loach DM, Alqumber M *et al.* D-alanyl ester depletion of teichoic acids in *Lactobacillus reuteri* 100-23 results in impaired colonization of the mouse gastrointestinal tract. *Environ Microbiol* 2007;**9**:1750–60.

Wang C-Y, Lin P-R, Ng C-C *et al.* Probiotic properties of *Lactobacillus* strains isolated from the feces of breast-fed infants and Taiwanese pickled cabbage. *Anaerobe* 2010;**16**:578–85.

Wegmann U, MacKenzie DA, Zheng J *et al.* The pan-genome of *Lactobacillus reuteri* strains originating from the pig gastrointestinal tract. *BMC Genomics* 2015;**16**:1023.

Wong ACN, Chaston JM, Douglas AE. The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *ISME J* 2013;**7**:1922–32.

Wong ACN, Luo Y, Jing X *et al.* The host as the driver of the microbiota in the gut and external environment of *Drosophila melanogaster*. *Appl Environ Microbiol* 2015;**81**:6232–40.

Wong ACN, Ng P, Douglas AE. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ Microbiol* 2011;**13**:1889–900.

Yarza P, Yilmaz P, Pruesse E *et al.* Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;**12**:635–45.

Yong JG, Lee JH, Hutkins RW. Functional analysis of the fructooligosaccharide utilization operon in *Lactobacillus paracasei* 1195. *Appl Environ Microbiol* 2007;**73**:5716–24.

Yuki N, Shimazaki T, Kushiro A *et al.* Colonization of the stratified squamous epithelium of the nonsecreting area of horse stomach by lactobacilli. *Appl Environ Microbiol* 2000;**66**:5030–4.

Zelante T, Iannitti RG, Cunha C *et al.* Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* 2013;**39**:372–85.

Zheng J, Ruan L, Sun M *et al.* A Genomic view of lactobacilli and pediococci demonstrates that phylogeny matches ecology and physiology. *Appl Environ Microbiol* 2015a;**81**:7233–43.

Zheng J, Zhao X, Lin XB et al. Comparative genomics Lactobacillus reuteri from sourdough reveals adaptation of an intestinal symbiont to food fermentations. *Sci Rep* 2015b;**5**:18234.

Zotta T, Ricciardi A, Parente E *et al.* Draft genome sequence of the respiration-competent strain *Lactobacillus casei* N87. *Genome Announc* 2016;**4**:e00348-16.

Table 1. Genomic and metabolic characteristics of species representing the different lifestyles of lactobacilli

Group	Organism	Habitat	OT^{a} (°C)	Genome size (Mb)	GC (%)	Lifestyle-associated traits	Mechanisms of host specificity	References
Free-living								
vac	*L. hokkaidonensis*	Grass/silage	25	2.3	38.1	pentose fermentation, aerotolerance	N/A	Tohno *et al* .(2013), Tanizawa *et al.* (2015)
buc	*L. buchneri*	Grass/silage	37	2.5	44.4	pentose fermentation, plant cell wall degradation	N/A	Heinl *et al.* (2012) *Kleinschmit et al.* (2006)
Nomadic								
pla	*L. plantarum*	Fruit flies; vertebrate digestive tract; plants and dairy products	37	3.2	44.5	bile resistance; metabolic versatility; two component systems.; extracellular proteins	N/A	Martino *et al.* (2016); Siezen *et al* (2010)
cas	*L.. casei*	raw and fermented dairy; silage, fermented vegetables, vertebrate digestive tract	30	2.8	46.5	metabolic flexibility; adhesion to intestinal villi; bile resistance; environmental sensing and adjustment; prototrophic to most amino acids	N/A	Cai et al (2007, 2009); Broadbent *et al.* (2012)
cas	*L. rhamnosus*	raw and fermented dairy, oral cavity, digestive tract of vertebrates, vagina	37	2.9	46.7	metabolic flexibility, fermentation of a wide range of carbohydrates; bile resistance; pili-mediated mucus adhesion; immunomodulation.	N/A	Douillard (2013,2013a); Ceapa (2015,2016);
Vertebrate-adapted								
sav	*L. ruminus*	Digestive tract; predominant in the bovine rumen; reported in humans, dogs, pigs, cats horses and primates.	37	2.1	43.5	bile and acid resistance; motility, substrate foraging; immunomodulation	Unknown	O'Donnell *et al*. (2015); Forde *et al.* (2011)
reu	*L. reuteri*	Proximal digestive tract; prevalent in rodents, pigs and chickens; reported in humans, dogs, minks, lambs, giraffes, cats and horses	37	1.9	38.6	bile and acid resistance; adhesion and biofilm formation	Epithelial adherence	Oh *et al* (2011); Frese *et al*. (2013)
del	*L. amylovorus*	Digestive tract; prevalent in swine; reported in chickens and horses.	37	2.0	37.8	bile and acid resistance; extracellular amylases, surface-attached "S-layers"; immunomodulation	Unknown	Kant *et al.* (2011); Grill *et al*. (2001)

sav	*L. salivarius*	Human oral cavity and digestive tract.; reported in breast milk and vagina and feces of pigs, raccoons, chickens and hamsters	37	2.0	32.5	bile resistance, bacteriocin production (Megaplasmid encoded)	N/A	Raftis *et at.* (2011, 2014); Li *et a.l* (2007)
del	*L. johnsonii*	Proximal digestive tract of rodents and poultry	37	1.8	34.5	Bacteriocin production and bile resistance	Unknown	Buhnik-Rosenblau *et al.* (2012); Pridmore (2004)
del	*L. iners*	Human vagina	37	1.3	32.5	Fe-S - defense against peroxide. Glycogen fermentation, adhesion	Epithelial adherence	Petrova *et al*. (2016); Macklaim *et al* (2011)
Insect-adapted								
del	*L. apis*	Bee	37	1.7	36.6	biofilm formation in the hindgut	Adherence/ Biofilm	Ellegaard *et al*. (2015); Anderson *et al.* (2013)
mel	*L. mellis*	Bee	30	1.8	36.2	putative exopolysaccharide formation, niche partition with other members of bee core microbiota	unknown	Ellagaard *et al*. (2015); Corby-Harris *et al*. (2014)
kun	*L. kunkeei*	Flowers, grapes, bees	30	1.5	36.4	fructophilic, resistant to phenolics and honey-desiccation	N/A	Vojvodic *et al*.(2013), Anderson *et al*. (2013), Endo *et al*. (2013) Maeno *et al* (2016)

vac, *L. vaccionostercus;* buc, *L. buchneri*; pla, *L. plantarum*; cas, *L. casei*; sav, *L. salivarius* ; reu, *L. reuteri* ; del, *L. delbrueckii* subsp. *delbrueckii*; mel, *L. mellifer*; kun, *L. kunkeei*

[a] Optimal growth temperature

FIGURE LEGENDS

Graphic abstract. Lifestyle of lactobacilli in a spectrum from free-living to strictly host-adapted species. Representative strains discussed in the text are placed respective to their lifestyle and order according to their reliance on environmental niches and the degree of host-specificity.

Figure 1. Word cloud representing the origin of lactobacilli. The words describe the origin of isolation of the type strains of lactobacilli; the square root of the font size of the words correlates to its frequency. Panel A. The origin of the 203 type strains of lactobacilli as described by Pot et al., (2014) or the species new description. The description was simplified as follows: All strains of human or animal origin are designated as human or animal, irrespective of the site of isolation; the origin of all isolates from cereal mashes used for production of alcoholic beverages are designated as "mash", the origin of all isolates from flowers or vegetable, sourdough, and silage fermentations were designated as "flower", "pickle", "sourdough" and "silage", respectively, irrespective of the plant species; the origin of all strains isolated from kimchi, sauerkraut, and fermented cabbage was designated as "sauerkraut"; the origin of isolates from various stages of beer, wine, and apple cider fermentation was designated as "beer", "wine", and "apple", respectively. The words "poultry" and "beef" represent meat; the words "chicken" and "cow" represent animals. Panel B. The origin of the same 203 type strains with a further simplification of the description of the origin as follows: the words representing spontaneous plant fermentations (pickle, sauerkraut and silage" was replaced by "plant"; the origin of all other food-associated organisms was omitted. The word cloud was generated with the online tool available at https://wordsift.org/

Figure 2. Core genome phylogenomic tree of *Lactobacillus sensu lato* (*Lactobacillus* spp. and *Pediococcus* spp.). The maximum likelihood tree was inferred by PhyML using the best model (LG+I+G+F) predicted by ProtTest and as described by Zheng *et al.* (2015a) with the inclusion of 18 additional species for which genome sequence data became available since

2015. *Eggerthia catenaformis* was used as an outlier for the phylogenetic analysis. The inner segments delineate homofermermentative and heterofermentative species, respectively. Members of the 24 phylogenetic groups of lactobacilli are indicated by the same color for branches and separated by dashed lines. The type strain of each group is printed in bold. Clusters in the *L. delbrueckii* and *L. salivarius* groups that differ in their ecology are separated by dashed lines. The solid circles in represent genome sizes of the type strains of each species; the area of the circle correlates with the genome size. Colour coding of the outer ring indicates the habitat or organisms where sufficient information is available. The habitat was assigned based on in depth phylogenetic and ecological studies as well as literature data related to the origin of isolation of the species; the assignment was additionally guided by database searches on www.imngs.org (Lagkouvardos et al. 2016; see Figure S1). Numbers indicates evolutionary transitions of lifestyle under the assumption of an ancestral free-living state, using an approach that is conceptually similar to that described by Sachs and co-workers (Sachs, Skophammer and Regus 2011)

Figure 3. Genomic and physiological characteristic of lactobacilli categorized by lifestyle. (A) Association between genome size and the number of coding sequences (CDSs). Information was obtain from the genomes of type strains (Table S1). Data points are color coded by lifestyle same as Figure 2. Model species discussed in the text are indicated. (B) Comparison of genome size (Mb) by lifestyle. (C) Comparison of GC content (%) by lifestyle (D) Comparison of optimal growth temperature (mean ± SD) by lifestyle; the special nomadic category of insect/associated is presented as a separate group. Box plots in panels B and C display the median, first and third quartile and the whiskers extend to the highest and lower values. Kruskal–Wallis with a Dunn's post hoc test was used to compare data between groups. Statistical significant groups are indicated (*, p<0.05; ***, p<0.001; ****, $p \leq 0.0001$). All analysis were performed in GraphPad Prism version 6.0 (GraphPad Software, La Joya, CA, USA).

Figure 4. Biofilms of lactobacilli on bee crop (A) and mouse forestomach (B) by confocal microscopy. Host tissue is colored green, lactobacilli are colored red.

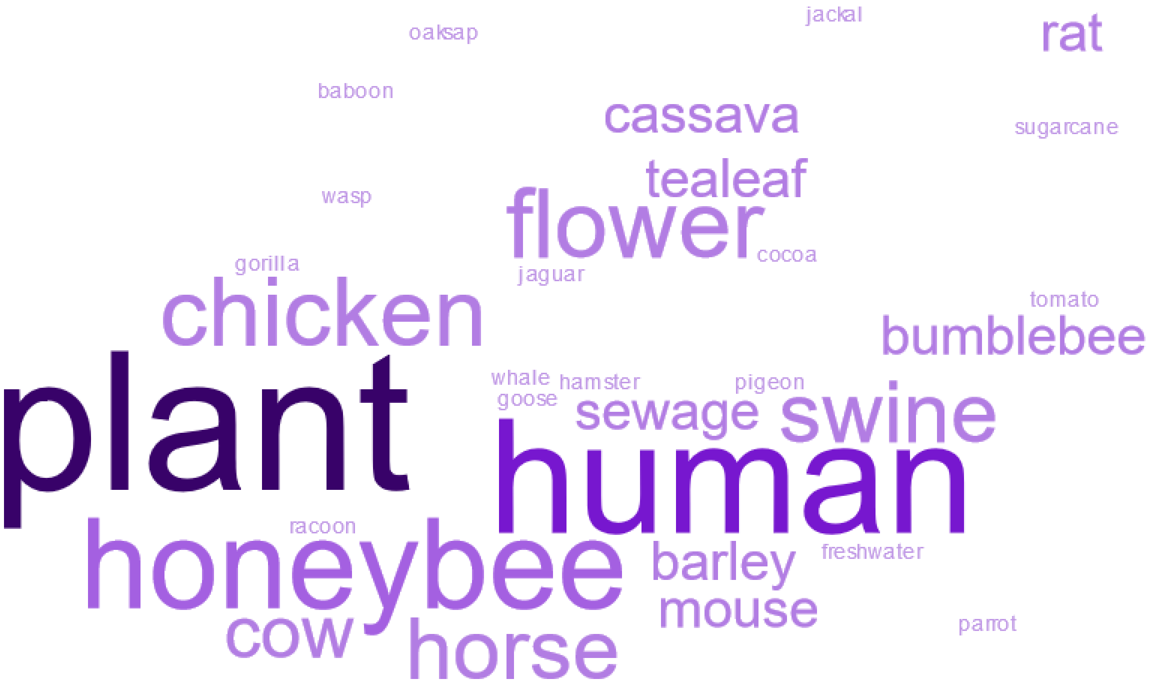
Figure 5. Maximum likelihood trees comparing the phylogenetic structure of the host-adapted species *L. reuteri* (A) and the nomadic *L. plantarum* (B). Tips of the branches are color coded by the strains' origin of isolation. The phylogeny of *L. reuteri* tree was inferred by multi locus sequencing analysis of 116 strains as described by Oh *et al.* (2010). Phylogeny of *L. plantarum* was constructed based on the core genome of 54 strains and as described in Martino *et al.* (2016). The degree of congruence between origin of isolation and phylogenetic clustering depicts the differences in lifestyle of these two species. *L. reuteri* strains cluster cohesively by host origin while *L. plantarum* strains display no obvious clustering by isolation source with isolates from the same source being scattered throughout the tree.

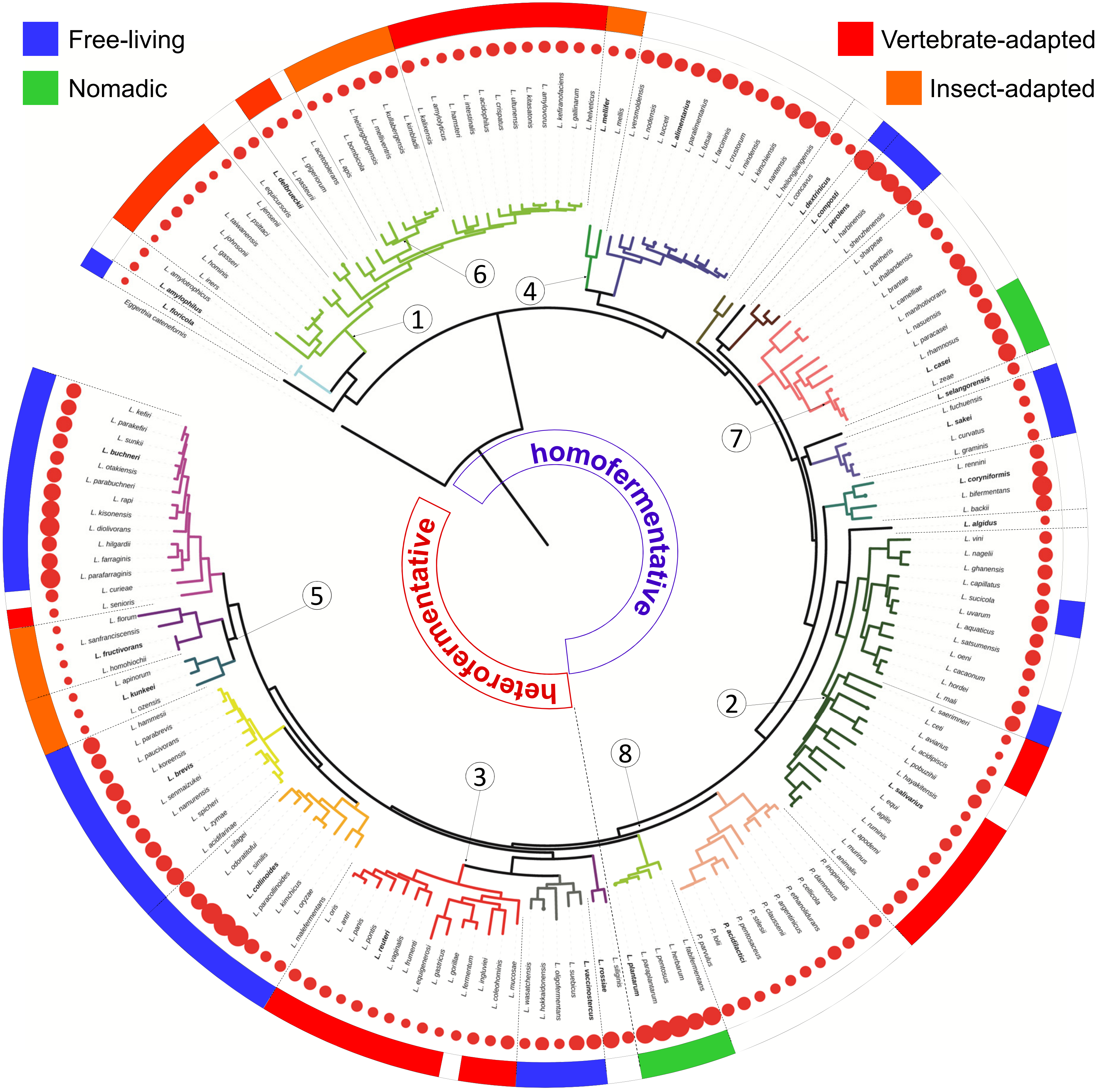
Figure 6. Model of the evolution of lifestyles in the genus *Lactobacillus*

A

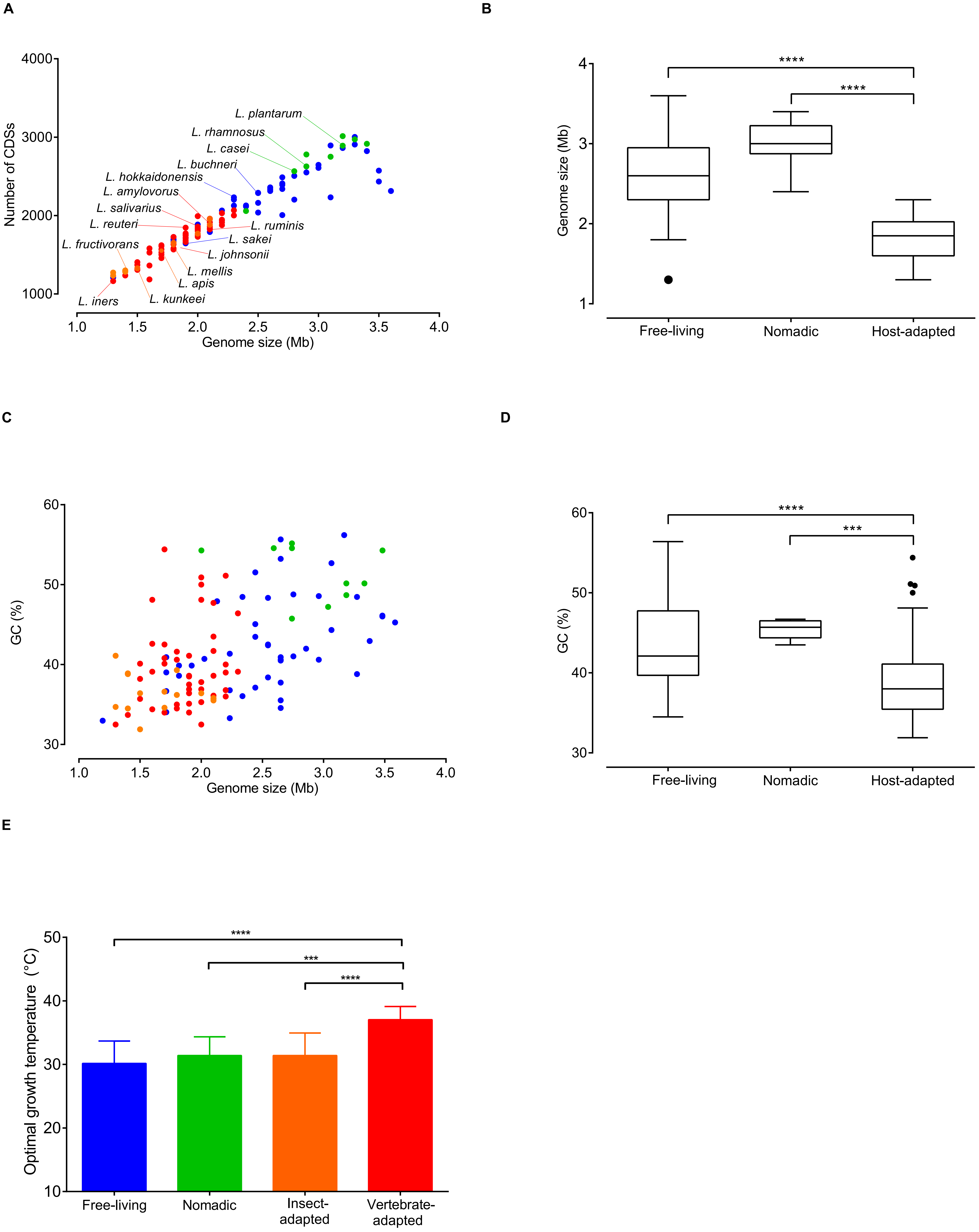

honeybee
rennin
sauerkraut
pickle
brewery
freshwater
milk
silage
apple
horse
sewage
mash
baboon
bumblebee
swine
tealeaf
poultry
tomato
breweryyeast
kefir
wine
fish
vinegar
jackal
chily
dairy
barley
rice
beer
cow
rat
mustard
human
flower
meat
oaksap
sausage
goose
cassava
beef
jaguar
mouse
cheese
chicken
soy
cocoa
sugarcane
wasp
hamster
corn
sourdough
lemonade
pigeon
tofu
whale
parrot
gorilla
racoon

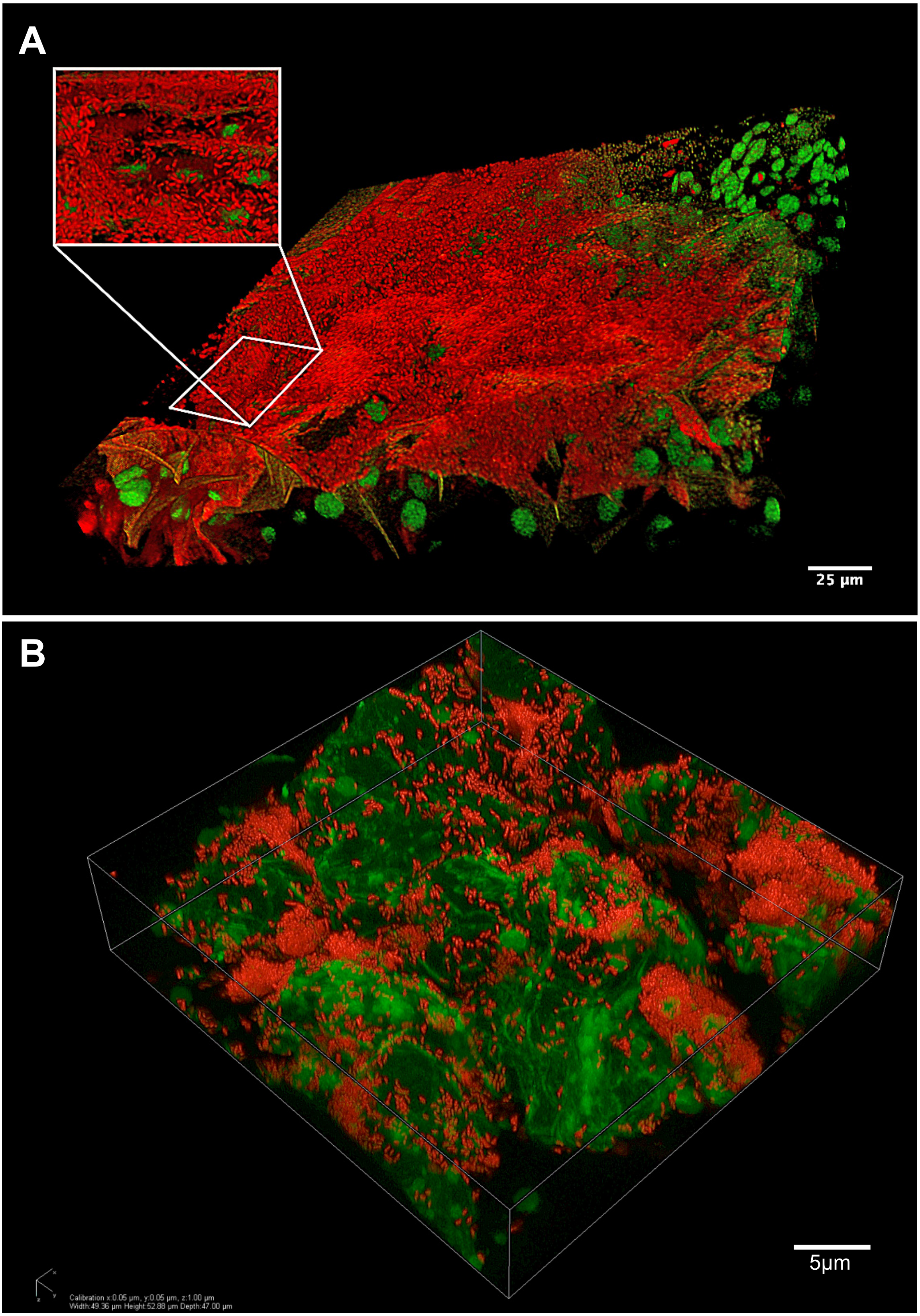
B

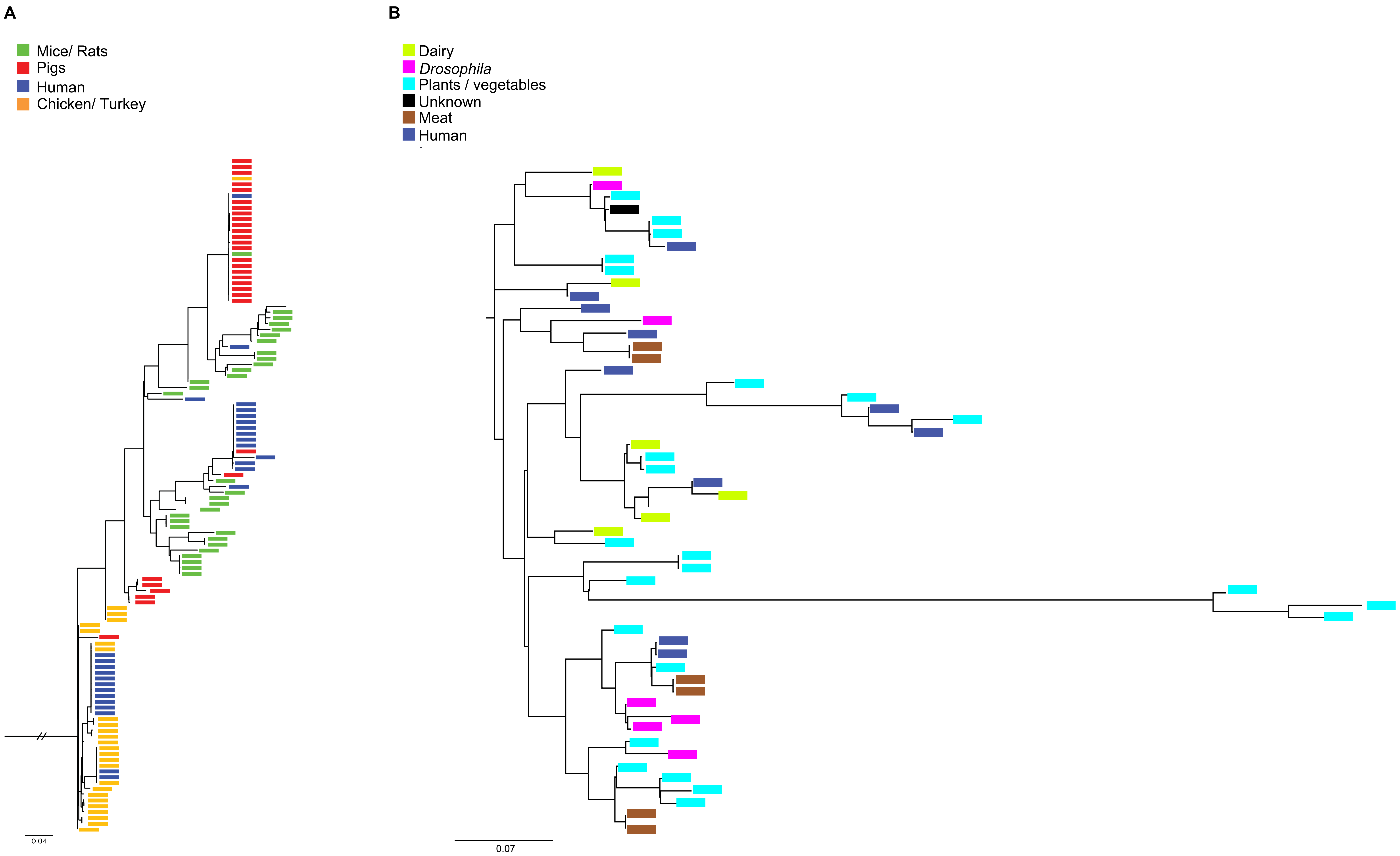

oaksap
jackal
rat
baboon
cassava
sugarcane
wasp
tealeaf
flower
cocoa
gorilla
jaguar
chicken
tomato
bumblebee
whale
hamster
pigeon
goose
sewage
swine
plant
human
racoon
freshwater
honeybee
barley
cow
mouse
horse
parrot

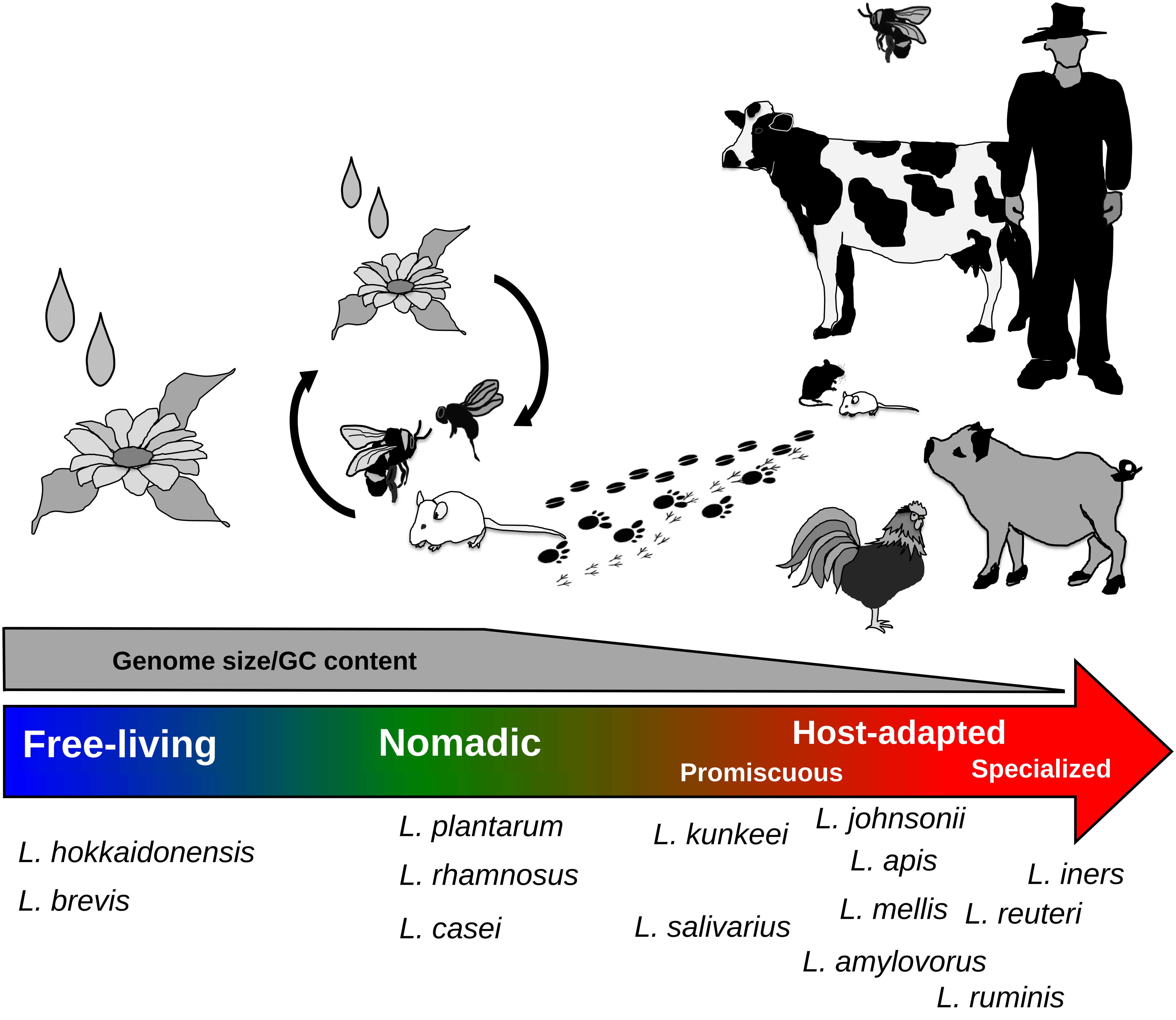


Free-living
Nomadic
Vertebrate-adapted
Insect-adapted
homofermentative
heterofermentative
1
2
3
4
5
6
7
8
Eggerthia catenaeformis
L. floricola
L. amylophilus
L. amylotrophicus
L. iners
L. hominis
L. gasseri
L. johnsonii
L. taiwanensis
L. psittaci
L. jensenii
L. equicursoris
L. delbrueckii
L. pasteurii
L. gigeriorum
L. acetotolerans
L. apis
L. bombicola
L. helsingborgensis
L. melliventris
L. kullabergensis
L. kimbladii
L. kalixensis
L. amylolyticus
L. hamsteri
L. intestinalis
L. acidophilus
L. crispatus
L. ultunensis
L. kitasatonis
L. amylovorus
L. kefiranofaciens
L. gallinarum
L. helveticus
L. mellifer
L. mellis
L. versmoldensis
L. nodensis
L. tucceti
L. alimentarius
L. paralimentarius
L. futsaii
L. farciminis
L. crustorum
L. mindensis
L. kimchiensis
L. nantensis
L. heilongjiangensis
L. concavus
L. dextrinicus
L. composti
L. perolens
L. harbinensis
L. shenzhenensis
L. sharpeae
L. pantheris
L. thailandensis
L. brantae
L. camelliae
L. manihotivorans
L. nasuensis
L. paracasei
L. rhamnosus
L. casei
L. zeae
L. selangorensis
L. fuchuensis
L. sakei
L. curvatus
L. graminis
L. rennini
L. coryniformis
L. bifermentans
L. backii
L. algidus
L. vini
L. nagelii
L. ghanensis
L. capillatus
L. sucicola
L. uvarum
L. aquaticus
L. satsumensis
L. oeni
L. cacaonum
L. hordei
L. mali
L. saerimneri
L. ceti
L. aviarius
L. acidipiscis
L. pobuzihii
L. hayakitensis
L. salivarius
L. equi
L. agilis
L. ruminis
L. apodemi
L. murinus
L. animalis
P. damnosus
P. inopinatus
P. cellicola
P. ethanolidurans
P. argentinicus
P. claussenii
P. stilesii
P. pentosaceus
P. acidilactici
P. lolii
P. parvulus
L. fabifermentans
L. herbarum
L. pentosus
L. paraplantarum
L. plantarum
L. siliginis
L. rossiae
L. vaccinostercus
L. suebicus
L. oligofermentans
L. hokkaidonensis
L. wasatchensis
L. mucosae
L. coleohominis
L. ingluviei
L. fermentum
L. gorillae
L. gastricus
L. equigenerosi
L. frumenti
L. vaginalis
L. reuteri
L. pontis
L. panis
L. antri
L. oris
L. malefermentans
L. oryzae
L. kimchicus
L. paracollinoides
L. collinoides
L. similis
L. odoratitofui
L. silagei
L. acidifarinae
L. zymae
L. spicheri
L. namurensis
L. senmaizukei
L. brevis
L. koreensis
L. paucivorans
L. parabrevis
L. hammesii
L. ozensis
L. kunkeei
L. apinorum
L. homohiochii
L. fructivorans
L. sanfranciscensis
L. florum
L. senioris
L. curieae
L. parafarraginis
L. farraginis
L. hilgardii
L. diolivorans
L. kisonensis
L. rapi
L. parabuchneri
L. otakiensis
L. buchneri
L. sunkii
L. parakefiri
L. kefiri


A
4000
3000
2000
1000
Number of CDSs
L. plantarum
L. rhamnosus
L. casei
L. buchneri
L. hokkaidonensis
L. amylovorus
L. salivarius
L. reuteri
L. ruminis
L. sakei
L. fructivorans
L. johnsonii
L. mellis
L. apis
L. kunkeei
L. iners
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Genome size (Mb)
B
4
3
2
1
Genome size (Mb)
****
****
Free-living
Nomadic
Host-adapted
C
60
50
40
30
GC (%)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
Genome size (Mb)
D
60
50
40
30
GC (%)
****
***
Free-living
Nomadic
Host-adapted
E
50
40
30
20
10
Optimal growth temperature (°C)
****
***
****
Free-living
Nomadic
Insect-adapted
Vertebrate-adapted


A
25 µm
B
5µm
x
y
z
Calibration x:0.05 µm, y:0.05 µm, z:1.00 µm
Width:49.36 µm Height:52.88 µm Depth:47.00 µm


A
🟩 Mice/ Rats
🟥 Pigs
🟦 Human
🟧 Chicken/ Turkey
0.04
B
🟨 Dairy
🟪 *Drosophila*
🟦 Plants / vegetables
⬛ Unknown
🟫 Meat
🟦 Human
0.07


Genome size/GC content
Free-living
Nomadic
Host-adapted
Promiscuous
Specialized
L. hokkaidonensis
L. brevis
L. plantarum
L. rhamnosus
L. casei
L. kunkeei
L. salivarius
L. johnsonii
L. apis
L. mellis
L. amylovorus
L. iners
L. reuteri
L. ruminis