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Making sense of the biodiversity and virulence of *Listeria monocytogenes*

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Abstract

Listeria monocytogenes is a foodborne pathogen responsible for listeriosis, an infection which can manifest in humans as bacteremia, meningoencephalitis in immunocompromised patients and the elderly, and fetal-placental infection in pregnant women. Reference strains from this facultative intracellular bacterium have been instrumental to investigate basic mechanisms in microbiology, immunology and cell biology. The integration of bacterial population genomics with environmental, epidemiological and clinical data allowed the uncovering of new factors involved in the virulence of *L. monocytogenes* and its adaptation to different environments. This review illustrates how these investigations have led to a better understanding of the bacterium's virulence and the driving forces that shaped it.

Highlights

- *Listeria monocytogenes* is a foodborne opportunistic bacterial pathogen
- Classical genetic approaches in reference strains allowed identification of core virulence genes
- Investigation of the species diversity of *L. monocytogenes* allowed uncovering hypo- and hypervirulent strains and new virulence genes
- Hypervirulent strains are host-associated, whereas hypovirulent strains are associated with food-processing environments
- Accumulation of loss-of-function mutations in virulence pathways leads to niche restriction and obligate saprophytism

Introduction

Listeria monocytogenes is the causal agent of listeriosis, a foodborne infection associated with high morbidity and mortality. The virulence of *L. monocytogenes* depends on its ability to colonize the gut and disseminate in the host. This Gram-positive bacterium was first isolated by G. Hülphers in Sweden, from rabbit liver necrosis [1] and from a human meningitis case in 1921 by J. Dumont [2]. It was then isolated and characterized by E.G.D. Murray in rabbits and guinea pigs from a breeding facility in the UK in 1926, and J.H. Pirie in wild gerbils in South Africa in 1927 [3, 4]. Human listeriosis is characterized by septicemia and central nervous system (CNS) infections, which mostly occur in immunocompromised and/or elderly individuals, and maternal-fetal infections, as well as rare anatomically localized infections [5-12]. *L. monocytogenes* can also induce gastroenteritis in otherwise healthy individuals when the ingested inoculum is large [13]. The foodborne origin of human listeriosis was formally proven in the 1980s [14]. Although listeriosis is relatively rare compared to other foodborne infections [15, 16], its high fatality rate (approximately 20% to 30%, despite effective antibiotic treatment [5]) makes it a significant public health concern [17]. Recently, the largest recorded outbreak occurred in South Africa, where 937 individuals were affected, with a 27% mortality rate [18]. *L. monocytogenes* has also been detected in the feces of healthy people, but the prevalence of *L. monocytogenes* fecal carriage in the general population is unknown [19, 20]. *L. monocytogenes* is therefore mostly considered as an opportunistic pathogen.

L. monocytogenes is not only an economical and public health issue, but is also a powerful model to study basic microbiology, host biology including innate and adaptive immunity and cell biology [21-24]. The identification of *L. monocytogenes* virulence factors in reference strains in cell culture models allowed deciphering the underlying mechanisms of its interactions with the host, and also addressing basic cell biology questions [22]. Further studies combining epidemiologic data with comparative genomics analyses of food- and clinically-associated strains allowed the uncovering of new genes involved in *L. monocytogenes* virulence [25]. Current investigations indicate that virulence factors are key for the adaptation of *L. monocytogenes* to its host and its optimal spread in the environment, and may modify our understanding of how this bacterium has acquired and selected these genes.

Uncovering virulence genes of *L. monocytogenes* by mutagenesis of reference strains

It was shown early on, using *in vitro* and *in vivo* approaches, that *L. monocytogenes* can enter macrophages, survive and be transferred to adjacent cells [26]. *In vivo* experiments in guinea pigs indicated that *L. monocytogenes* can be released in the cytoplasm of intestinal epithelial cells, surrounded by electron dense material [27], which would later come to be identified as polymerized actin. Entry of *L. monocytogenes* in non-phagocytic epithelial cells was reported *in vitro* in the human enterocyte-like cell line Caco-2 [28]. Most of the factors involved in key phenotypes of the cellular microbiology of *L. monocytogenes*, namely entry into host cells, escape from the internalization vacuole and spread from cell to cell, were characterized in the 1980s and 1990s and shown to be critical for virulence *in vivo*. They were mostly discovered by a combination of molecular and cell biology approaches, using transposon bacterial mutagenesis, gene knock-out and complementation studies coupled with infection of cultured macrophages and epithelial cell lines. These factors were characterized in the laboratory reference strains EGD, 10403S or EGDe, which all belong to the serovar 1/2a but to two distinct lineage II clonal complexes (CCs; see below). EGD (CC7) derives from a strain isolated in guinea pig by E. G. D. Murray, hence its name [3]. 10403S (CC7) is a streptomycin-resistant strain derived from 10403, a strain isolated from a human listeriosis case [29]. The origin of EGDe (CC9) is more difficult to trace. It was supposed to be an EGD isolate, but genome analyses have revealed that both strains are actually unrelated [30, 31].

LIPI-1 genes

A phenotypic characteristic of *L. monocytogenes* is its ability to completely lyse red blood cells (beta hemolysis), due to the expression of a hemolysin encoded by *hly* and named listeriolysin O (LLO) [32, 33]. Screening and characterization of nonhemolytic mutants obtained by transposon mutagenesis indicated that hemolysin expression is critically needed for *L. monocytogenes* virulence [34]. Further characterization of Δ*hly* mutant in intestinal Caco-2 cells showed that LLO's pore-forming toxin is involved in escape of *L. monocytogenes* of its internalization vacuole [28]. Later analysis involved the characterization of two phospholipases, PlcA and PlcB (activated by the metalloprotease Mpl), which are also involved in vacuolar escape [35, 36]. Once in the cytosol, *L. monocytogenes* can polymerize actin and form so-called “actin comet tails” via its surface protein ActA, which propel *L. monocytogenes* in the cytoplasm, and allow its spread from cell to cell [37, 38]. The study of *actA* and *plcA* transposon and deletion mutants indicated that they are both critical for *L. monocytogenes* virulence [39, 40]. Analysis of an avirulent non-hemolytic variant of the NCTC 7973 guinea pig strain (isolated by E.G.D. Murray) allowed the characterization of a

transcription factor involved in *hly* expression which was named PrfA for "Positive regulatory factor of listeriolysin (*lisA*, the other designation for *hly*)" [41, 42]. PrfA was further shown to be the major regulator of *L. monocytogenes* virulence genes *in vivo*, including *prfA*, *hly*, *actA*, *plcA*, *mpl*, *plcB* and the nucleomoduline *orfX* [43], which are all encoded by the Listeria pathogenicity island 1 (LIPI-1) [44] (Figure 1A). PrfA is also transcriptionally regulated by σ^B, that controls the general stress response in *L. monocytogenes*, is regulated post-transcriptionally and requires a glutathione co-factor for full activation [45, 46].

inlAB

Transposon mutagenesis was also used to screen for mutants that are unable to enter Caco-2 cells. This approach allowed for the identification and characterization of internalin (InlA), a leucine-rich repeats (LRR)-containing protein anchored to the bacterial peptidoglycan by a Leu-Pro-X-Thr-Gly (LPXTG) motif [47]. The receptor of InlA, namely E-cadherin (Ecad), which is expressed on epithelial cells and forms *adherens* junctions, was identified by affinity chromatography [48]. Further analyses indicated that the InlA-Ecad interaction is species-specific, as mouse and rat Ecad are not a receptor for InlA, in contrast to human Ecad. Indeed, InlA interaction with Ecad requires a proline in the 16th amino acid position of Ecad, which is present in permissive species (human, rabbit, guinea pig, gerbil), and is replaced by a glutamic acid in non-permissive murine species (mouse, rat), preventing InlA interaction with Ecad in the latter [49]. In guinea pigs and transgenic mice expressing human Ecad, InlA-Ecad interaction is critical for *L. monocytogenes* to cross the intestinal barrier, at the villus level, by transcytosis across goblet cells [50, 51]. Live imaging in intestinal organoids has now shown that *L. monocytogenes* hijacks E-cadherin recycling for its: (*i*) entry into; (*ii*) transepithelial transfer across; and (*iii*) exocytosis from goblet cells, in a dynamin-, microtubule- and Rab11-dependent manner, respectively [52]. *inlA* is located in an operon comprising a downstream gene called *inlB* [47]. InlB is also an LRR-containing protein; however, it is not covalently linked to the bacterial surface by an LPXTG motif, but loosely attached to it by its Gly-Trp (GW) motif. InlB mediates the entry of *L. monocytogenes* in cultured cells by interacting with c-Met/HGFR, which is ubiquitously expressed [53, 54]. InlB-c-Met interaction is also species-specific: it occurs in humans, mice and gerbils but not in rabbits nor guinea pigs [55]. InlB is not involved in *L. monocytogenes* crossing of the intestinal barrier [55]. In order to study the role of the InlA-Ecad and InlB-c-Met interactions in organs other than the intestine, knock-in mice KIE16P, in which the glutamic acid in 16th position of the Ecad is replaced by a proline, were generated [56]. In contrast to the intestinal barrier where only InlA is

involved, InlA and InlB are both required and act in a conjugated manner in *L. monocytogenes* crossing of the placental barrier in gerbils and in KIE16P mice [56, 57].

Uncovering virulence genes of *L. monocytogenes* by comparative *Listeria* genomics

While classical genetics has identified major virulence factors in *L. monocytogenes*, whole genome sequencing of pathogenic bacterial species, which was initiated with *Mycoplasma genitalium* [58], opened a new avenue for identifying genes involved in virulence, by comparison of the genomic content of pathogenic and phylogenetically closely related non-pathogenic bacterial species. Indeed, comparison of the genome sequences of *L. monocytogenes* EGDe and the nonpathogenic *Listeria innocua* CLIP 11262 reference strains allowed identification of putative virulence factors present in *L. monocytogenes* but absent in *L. innocua* [59] (Figure 1B).

Sequencing of EGDe revealed the presence of 25 genes that code for proteins of the internalin family, characterized by the presence of an LRR domain. Seventeen of them had not been described before, including InlJ, an LPXTG anchored protein involved in virulence, whose exact function remains to be determined [60, 61] and InlP (*lmo2470*), a secreted protein involved in the infection of the placenta, among other organs [62, 63].

Comparative genomics also revealed the presence of GW motif-containing proteins other than InlB in EGDe (and which were absent in *L. innocua* CLIP 11262) that are involved in virulence. As an example, the GW-containing protein Auto is an autolysin involved in cell entry and virulence [64].

Another gene that is absent in *L. innocua,* named *bsh,* has been identified by this strategy. This PrfA-regulated virulence gene encodes for a bile salt hydrolase, which increases resistance of *L. monocytogenes* to bile, and enhances liver infection and bacterial faecal carriage [65], an essential property for a foodborne pathogen.

Intraspecies genomic studies and identification of novel virulence genes

The biodiversity of L. monocytogenes

The biodiversity of *L. monocytogenes* was first investigated via serotyping, based on *Listeria* somatic and flagellar antigens, which allowed identifying the four main serotypes (1/2a, 1/2b, 1/2c and 4b) associated to listeriosis [66]. However, this method does not allow establishing reliable phylogenetic links between strains. Multilocus enzyme electrophoresis differentiated two main lineages, I and II [67], while gene variation studies and multi-locus genotyping assays have led to the delineation of two minor lineages III and IV [68, 69]. In order to

179 determine the population structure of *L. monocytogenes*, multi-locus sequence typing
180 (MLST), allowing to translate sequence diversity into numerical profiles, called sequence
181 types (STs), was set up with seven housekeeping genes [70]. Using this technique, isolates
182 having at least 6 alleles in common out of these seven genes are grouped into the same clonal
183 complex (CC). With advances in whole genome sequencing, this method has been extended
184 to larger sets of loci (typically thousands) to allow strain differentiation at the level of the
185 whole- or core genome (wgMLST or cgMLST, respectively) [71-75]. A standard
186 nomenclature system has been proposed based on the cgMLST scheme of 1,748 core loci
187 [75]. Isolates sharing less than 150 and 7 allelic mismatches are grouped into sublineages
188 (SL) and cgMLST types (CT), respectively, which allows for efficient inter-laboratory
189 communication, in particular for emerging strain types [76] (Figure 2A). In addition, the
190 compilation of genotypic data in dedicated databases, such as BIGSdb-*Lm*
191 (http://bigsdb.pasteur.fr/listeria) or GenomeTrakr (hosted at
192 https://www.ncbi.nlm.nih.gov/pathogens) allow for in-depth studies of the global spread and
193 biodiversity of *L. monocytogenes*.
194
195 *Hypervirulent strains belong to distinct CCs compared to hypovirulent strains*
196 *L. monocytogenes* can be divided into four evolutionary lineages [77], 13 serotypes [78]
197 grouped into 6 genoserogroups [79] and more than 170 clonal complexes and 300 sublineages
198 (http://bigsdb.pasteur.fr/listeria). Of these, lineage I (serotypes 4b and 1/2b) and II (serotypes
199 1/2a and 1/2c) isolates represent the majority of clinical isolates. The most prevalent CCs and
200 SLs are geographically and temporally widespread [70, 80, 81]. However, an uneven
201 prevalence is observed among food *vs*. clinical cases [25, 75, 80], as previously noted at the
202 lineage and serotype levels [77]. While lineage II, in particularly sublineages SL9 (CC9) and
203 SL121 (CC121) are mostly associated with food, lineage I isolates, and in particular
204 sublineages SL1 (CC1), SL2 (CC2), SL4 (CC4) and SL6 (CC6) are strongly associated with
205 clinical cases in the Western world [25, 75, 82-87] (Figure 2B). In Asia, specifically in China
206 and Taiwan, the most prevalent sublineage in both food and clinical cases is SL87 (CC87,
207 lineage I, serotype 1/2b) [84, 88, 89], suggesting that food habits and/or local specificities are
208 involved in geographic diversity of *L. monocytogenes*.
209 A large study of 6,663 *L. monocytogenes* isolates of clinical and food origins prospectively
210 collected in France between 2005 and 2013 reported a higher prevalence of CC9 and CC121
211 (both of lineage II) isolates in food samples, while CC1, CC6, CC2 and CC4 (all of lineage I)
212 were the most prevalent in clinical cases [25]. In addition, CC1 and CC4 were found to be

statistically associated with more invasive forms of listeriosis (maternal-neonatal and CNS infections). Similar results have been observed within farm environments, with CC1 being also associated with CNS infection in ruminants in Switzerland [90]. This could reflect a difference in virulence and/or in exposure of at-risk patients to these strains. Association of CCs to clinical cases is inversely correlated to patients' immunosuppressive comorbidities, suggesting that these are more virulent than CCs most associated with food [25]. *In vivo* studies, which compared CC1, CC4, CC6, CC9 and CC121 isolates and the laboratory reference strains EGDe and 10403S in a mouse model permissive for oral *L. monocytogenes* infection, unambiguously showed that strains from CCs most associated with clinical cases (CC1, CC4 and CC6) are hypervirulent when compared to laboratory reference strains EGDe and 10403S, as they induced a more pronounced body weight decrease and are more neuroinvasive [25]. In contrast, CC9 and CC121 are hypovirulent when compared to these laboratory reference strains. Of note, CC9 and CC121 are not fully avirulent, since they can still infect laboratory animals, although to a lower degree, as well as immunodeficient patients [25]. This heterogeneity in virulence among lineages and CCs led to an investigation of the putative molecular mechanisms involved in both hypo- and hyper-virulence.

Virulence factors found in the core genome

While *inlA* is present in all *L. monocytogenes* strains, some strains, including the reference laboratory strain LO28 (CC9, lineage II), express a truncated and therefore nonfunctional InlA [91]. Epidemiological analysis indicated that clinical strains, and most significantly those from maternal-fetal infections, express full-length InlA more frequently than strains from food origins [70, 92]. InlA is truncated in the majority of hypovirulent CC9 and CC121 isolates as well as in other lineage II clonal complexes (e. g. CC331, CC199 and CC321[75]). Hypovirulence can therefore, at least in part, be accounted for by InlA truncation, leading to decreased translocation across the intestinal barrier and placental infection [50, 56, 93].

Similarly to *inlAB*, LIPI-1 genes are present in all *L. monocytogenes* SLs and are therefore core virulence genes (Figure 1A). As indicated above, non-pathogenic *L. innocua* lacks LIPI-1 and *inlAB* genes, however rare *L. innocua* strains contain both LIPI-1 and *inlA* that are functional *in vitro* and *in vivo* [94-99]. This indicates that *L. monocytogenes* and *L. innocua* species likely evolved independently from a common pathogenic ancestor harboring both the LIPI-1 and *inlAB* virulence loci. Speciation likely occurred upon differential environmental selective pressures, with *L. innocua* evolving in an environment where virulence genes are not needed [100]. In contrast, 0.1% of *L. monocytogenes* strains from both lineage I and II are

non-hemolytic. 93.3% of these non-hemolytic strains harbor at least one loss-of-function mutation in *prfA,* and the remaining 6.7% harbor *hly* mutations leading to LLO inactivation. In both of these cases, this results in a strong decrease in virulence, suggesting that niche restriction leading to sub-speciation may still occur within *L. monocytogenes* species [101].

Virulence factors encoded by the accessory genome

LIPI-3

The LIPI-3 virulence cluster, which is absent in *L. monocytogenes* reference strains but present in lineage I F2365 (SL1/CC1) and H7858 (SL6/CC6) outbreak strains, has also been characterized (Figure 1A). It consists of 8 genes, including a hemolytic and cytotoxic factor, listeriolysin S (LLS), involved in virulence [102]. LLS, encoded by *llsA*, acts as a bacteriocin and alters microbiota composition, resulting in an increase in the luminal amount of *L. monocytogenes*, while *llsB*, another gene of LIPI-3, plays a role in the systemic phase of the infection [103, 104]. Functional LIPI-3 or its remnants can also be present in some *L. innocua* strains [98, 105], where its role is not defined, reinforcing the hypothesis that the two species share a common pathogenic ancestor. The presence of LIPI-3 in *Lm* sublineages SL1 (CC1), SL4 (CC4) and SL6 (CC6), which are the most associated with clinical cases [75, 98], and its absence in lineage II strains, suggests a possible role in their hypervirulence.

LIPI-4

Comparative genomic analyses derived from biodiversity studies allowed the characterization of LIPI-4, a putative phosphotransferase system (PTS) present in SL4, a sublineage of the lineage I, which strongly associated with a clinical origin (Figure 2B), particularly in CNS and placental infections [25]. LIPI-4 is involved in placental and CNS infection in an *in vivo* model of listeriosis, by a so far unknown mechanism (Figures 1A and 2A) [25]. Interestingly, LIPI-4, is also present in the *L. monocytogenes* sublineage that is the most prevalent in Asia, SL87 (CC87) [106], as well as in *L. innocua* [98]. Its actual role in such a non-virulent species is currently unknown.

LIPI-2

LIPI-2 is a genomic region discovered in *Listeria ivanovii,* a *Listeria* species which is pathogenic for mammals, mainly ovines and bovines, but also rarely humans [108, 109]. LIPI-2 was first described as an *L. ivanovii*-specific genomic region that encodes a sphingomyelinase involved in phagosome disruption, as well as 10 genes of the internalin

family [110]. This region is involved in *L. ivanovii* virulence, together with the *L. ivanovii* LIPI-1 orthologous region, both *in vitro* and in an *in vivo* sheep model of infection [110]. *L. monocytogenes* isolates belonging to a new sublineage of the major lineage II (SL626/CC33, serovar 4h) recovered from an ovine listeriosis outbreak in China have been characterized as hypervirulent [107]. These isolates contain a partial LIPI-2 locus, comprising only the sphingomyelinase *smcL* and the internalins *i-inlF* and *i-inlE* genes (Figure 1A), likely by acquisition of exogenous DNA from *L. ivanovii*. The specific role of the partial LIPI-2 in hypervirulence of this newly described *L. monocytogenes* sublineage remains to be elucidated.

Heterogeneity in virulence as a reflection of differential host adaptation?

The distribution of *L. monocytogenes* is wide. It has been isolated in humans and animals in all continents except Antarctica [80]. Linked to its ability to tolerate extreme temperatures, acidity and osmolarity, *L. monocytogenes* can persist and grow in soil, plants, water, food and silage [111-113]. However, the distribution of *L. monocytogenes* lineages and CCs is not homogeneous in these ecosystems, indicating that CCs emergence may be driven by their selective adaptation to the environment in which they are the most abundant [25, 77].

When investigating food samples, hypervirulent CC1, CC4 and CC6 were found to be statistically associated with dairy products, whereas hypovirulent *L. monocytogenes* clones, CC9 and CC121 were found associated with meat products [114], suggesting a differential adaptation of the CCs to their environment. Interestingly, CC9 and CC121 harbor genes involved in tolerance to benzalkonium chloride, a surface disinfectant commonly used in food production environments, and are more efficient than their hypervirulent counterparts to form biofilms in the presence of this disinfectant [114, 115]. Other stress resistance genes such as the SSI-1 and SSI-2 stress survival islets [116, 117] are also typically present in lineage II, and contribute to *L. monocytogenes* survival and growth in the stress conditions encountered in food and food processing environments (e.g. high salt concentrations, oxidative stress). Lineage I isolates typically display higher tolerance to low pH [118], which can be advantageous for the survival of *L. monocytogenes* in the host's gastric acidic environment. Consistent with these observations, strains of hypervirulent CCs colonize the gut more efficiently than those of hypovirulent CCs [114], which may account for their strong association with ruminant feces [119] and dairy products, which may result from milk fecal contamination [114] (Figure 3).

Concluding remarks

L. monocytogenes is a major model pathogen which has allowed scientific breakthroughs in microbiology, immunology, cell biology and host-pathogen interactions. It has also emerged as a powerful model to study how microbes evolve from their natural habitat to anthropic environments. While advances have been made in the discovery of genetic factors involved in virulence and adaptation to host *vs*. food processing environments, the mechanisms by which *L. monocytogenes* adapts to various environments remains to be fully deciphered. Seminal studies have indicated how the transcriptome in the EGDe reference strain responds to the switch from saprophytism to virulence [120]. Future large-scale transcriptomics and metabolomics studies with representative strains from different clonal complexes will enrich our understanding of how *L. monocytogenes* can adapt to the environments in which it thrives. Further studies will also be needed to decipher the forces driving hypervirulence and its relationship with adaptation to the host, and how this relates to capacity of *L. monocytogenes* to be shed back in the environment and transmitted to new hosts, as suggested by data from ruminants and animal models [114, 119]. The continuous analysis of biodiversity of *L. monocytogenes*, together with environmental, epidemiological, clinical and experimental data obtained from genome wide association studies will shed light into the mechanisms and selective pressures that drive *Listeria* adaptation to diverse environments.

Figure Legends

Figure 1. Virulence genes of *L. monocytogenes*

A) Typical genetic organization of *Listeria* pathogenicity islands (LIPI; colored arrows). Flanking genes (gray arrows) are labeled based on their orthologs in *L. monocytogenes* EGDe, when existing. *Lm, L. monocytogenes; Lin, L. innocua; Liv, L. ivanovii;* *, LIPI-2 genes present in Liv aatypical *L. monocytogenes* CC33 isolates are shown in darker yellow.

B) Genome maps of *L. monocytogenes* EGDe and *L. innocua* CLIP11262 [59]. Inner circles represent the GC skew ((G − C)/(G + C)) and GC content ((G+C)/(G+C+A+T)) of *L. monocytogenes* EGDe. Gaps in *L. innocua* chromosome (blue) represent genomic regions absent in *L. monocytogenes* genome (red). The genomic positions of known *L. monocytogenes* virulence genes are shown and their common and/or locus names indicated. Internalin genes are highlighted in red. Triangles denote the genomic positions of LIPI regions in either EGDe (black triangle) or other *Listeria* species (open triangles), as detailed in panel A.

Figure 2. Population structure of *L. monocytogenes*

A) Population structure of *L. monocytogenes* based on cgMLST profiles (adapted from [75]; *N*=1,696 isolates collected from food and clinical samples, mostly in North America and Europe). Branches are colored by phylogenetic lineage (I-IV, shown in red, blue, green and gray, respectively) and collapsed by sublineage (SL). The vertical dashed bar indicates the established cgMLST cut-off to define SLs (i.e. 150 allelic differences out of 1748 loci). Main SLs are labeled on the branches and corresponding MLST clonal complexes (CCs) are shown in the first column, PCR serogroups, source types and continents of isolation are colored according the key panels (upper left). Dark blue boxes denote the presence of selected genetic traits: pathogenicity islands (LIPI-1 to -4), genes involved in teichoic acid biosynthesis (*gltAB*, *tagB* and *gtcA*), genes coding for internalins (*inlABCEFGHJK*), autolysins (*aut* and *autIVb)* and bile resistance (*bsh*), genes involved in benzalkhonium resistance (*bcrABC* operon*, ermC*, *Tn6188::ermC* and *ermE)*, stress resistance islets (SSI-1 and SSI-2) and biofilm formation and virulence (*comK)*.

B) Frequency of the most prevalent *L. monocytogenes* MLST clonal complexes in clinical (*y*-axis) *versus* food (*x*-axis) isolates, based on a exhaustively collected isolates dataset obtained in the scope of listeriosis surveillance (*N*=6,633 isolates, France 2005-2013). (reproduced with permission from [25]). Each circle represents a clonal complex, and is colored according to its lineage. The size of each circle is proportional to the number of isolates it represents. The clonal complexes of common reference lab strains (CC7: strains EGD and 10403S; CC9: EGDe and LO28), are also indicated.

Figure 3. Trade-off between host-associated and environmental lifestyles of *L. monocytogenes*

Properties of *L. monocytogenes* strains adapted to a host environment (in red) *versus* of those adapted to saprophytism (natural and food-processing environments; in blue). Listeriosis results from the interplay between host and microbial factors: the less immunocompromised is the host, the more virulent *L. monocytogenes* strains need to be to cause disease. Thus, immunocompromised hosts tend to be susceptible to even saprophytic (hypovirulent) *L. monocytogenes*, whereas healthy hosts are usually only susceptible to host-associated (hypervirulent) strains, as shown in [25].

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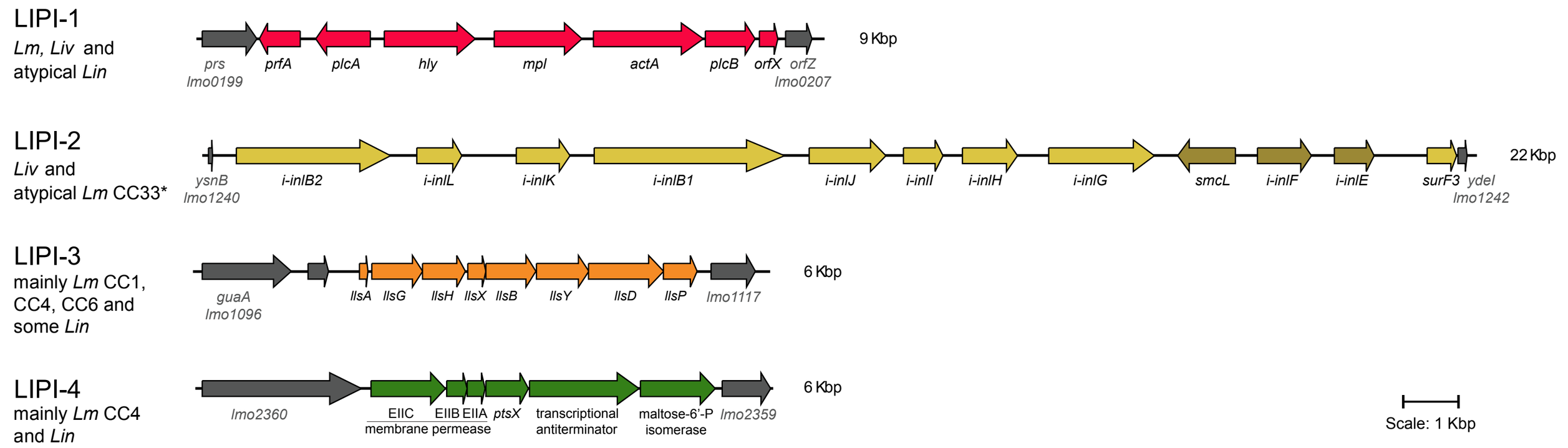
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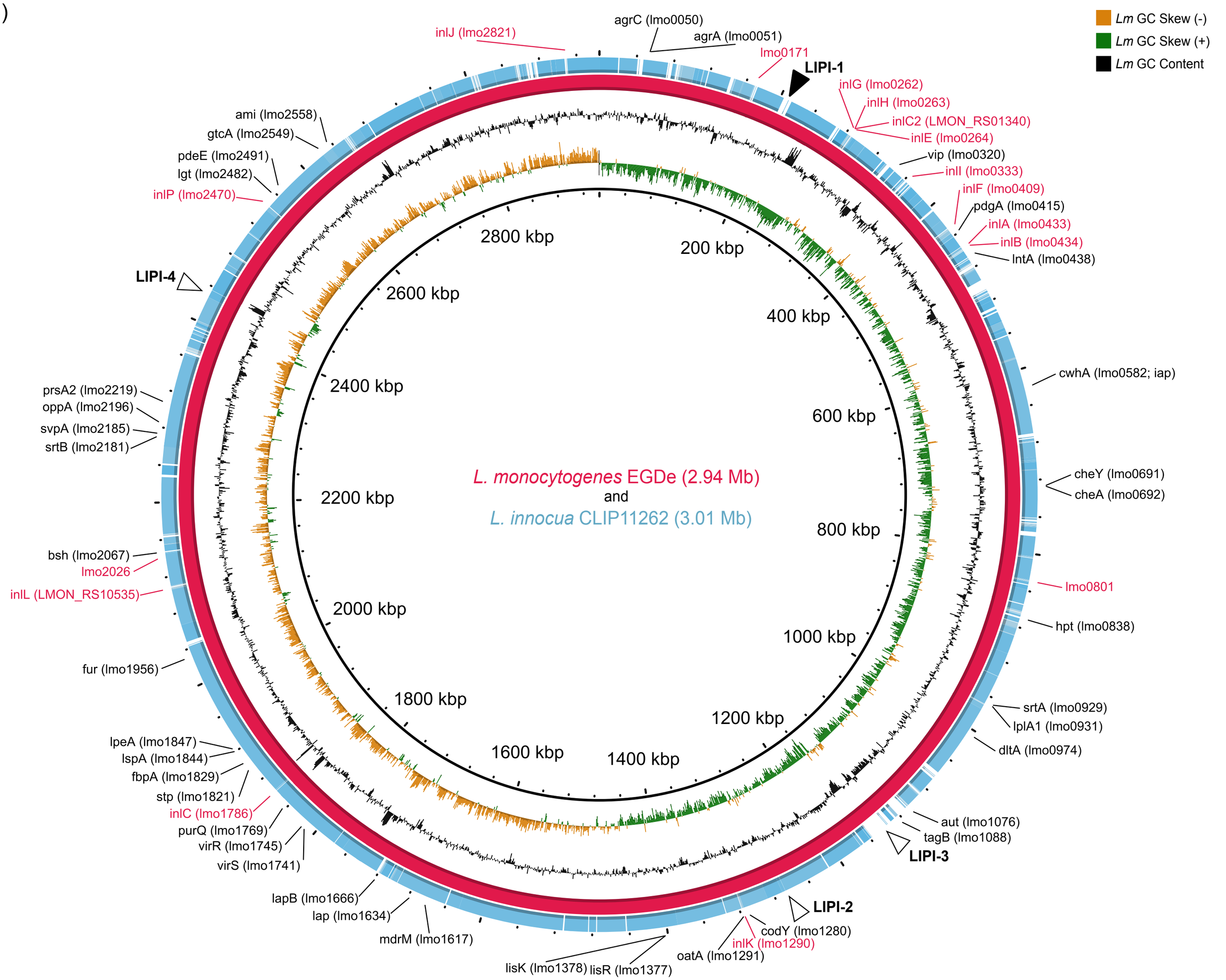
Outstanding questions

- What are the natural environments that have led to the current biodiversity of *L. monocytogenes*?
- What is the primary function of LIPI-1 genes, and other so-called “virulence genes”?
- What are the driving forces that have selected the virulence genes of *L. monocytogenes*?
- How does *L. monocytogenes* maintain its virulence since disease is not implicated in inter-host transmission?

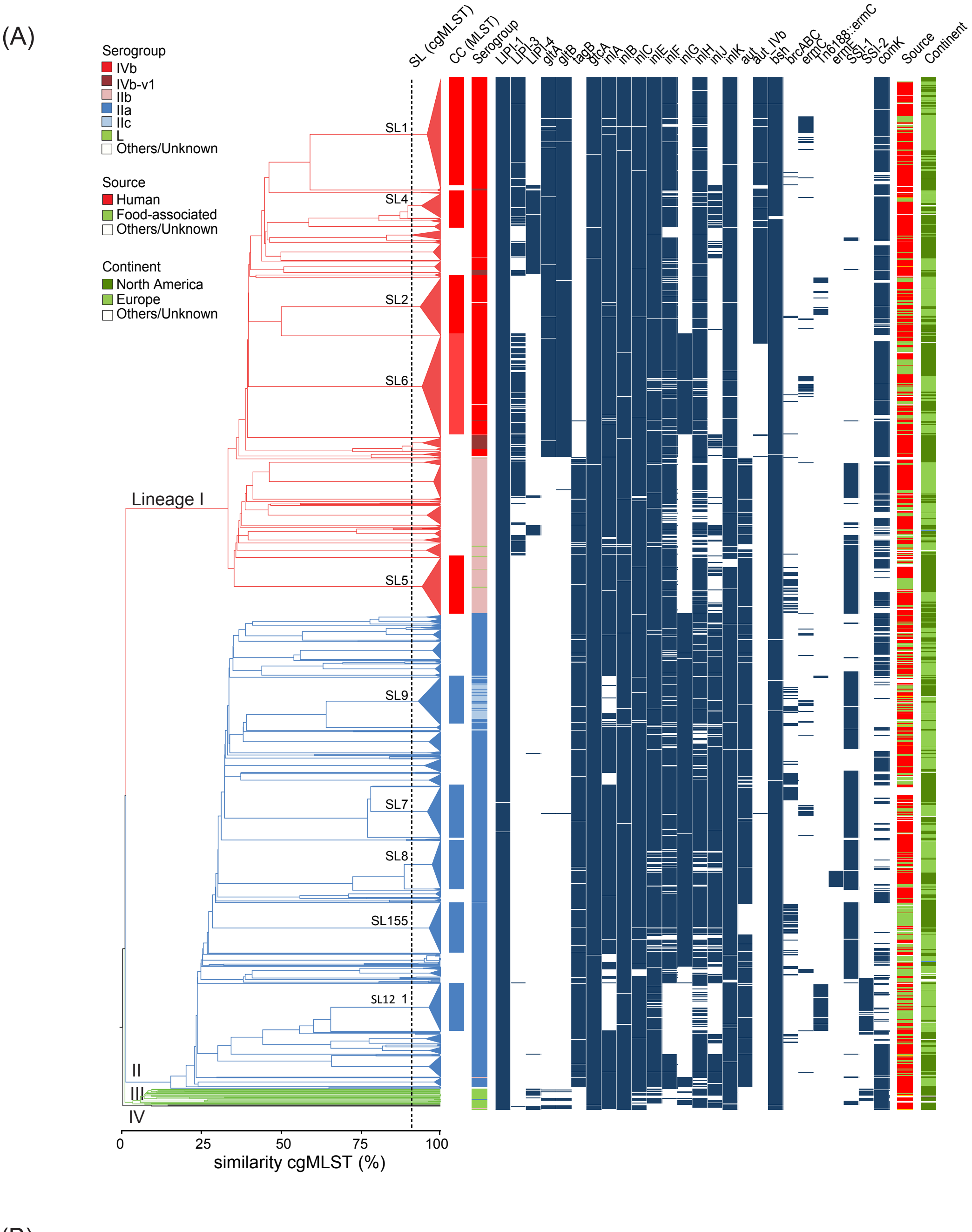
(A)


LIPI-1
Lm, Liv and atypical Lin
prs lmo0199
prfA
plcA
hly
mpl
actA
plcB
orfX
orfZ lmo0207
9 Kbp
LIPI-2
Liv and atypical Lm CC33*
ysnB lmo1240
i-inlB2
i-inlL
i-inlK
i-inlB1
i-inlJ
i-inlI
i-inlH
i-inlG
smcL
i-inlF
i-inlE
surF3 ydeI lmo1242
22 Kbp
LIPI-3
mainly Lm CC1, CC4, CC6 and some Lin
guaA lmo1096
llsA
llsG
llsH
llsX
llsB
llsY
llsD
llsP
lmo1117
6 Kbp
LIPI-4
mainly Lm CC4 and Lin
lmo2360
EIIC membrane permease
EIIB EIIA
ptsX
transcriptional antiterminator
maltose-6'-P isomerase
lmo2359
6 Kbp
Scale: 1 Kbp

(B)


Lm GC Skew (-)
Lm GC Skew (+)
Lm GC Content
L. monocytogenes EGDe (2.94 Mb)
and
L. innocua CLIP11262 (3.01 Mb)
200 kbp
400 kbp
600 kbp
800 kbp
1000 kbp
1200 kbp
1400 kbp
1600 kbp
1800 kbp
2000 kbp
2200 kbp
2400 kbp
2600 kbp
2800 kbp
LIPI-1
LIPI-2
LIPI-3
LIPI-4
agrC (lmo0050)
agrA (lmo0051)
lmo0171
inlG (lmo0262)
inlH (lmo0263)
inlC2 (LMON_RS01340)
inlE (lmo0264)
vip (lmo0320)
inlI (lmo0333)
inlF (lmo0409)
pdgA (lmo0415)
inlA (lmo0433)
inlB (lmo0434)
lntA (lmo0438)
cwhA (lmo0582; iap)
cheY (lmo0691)
cheA (lmo0692)
lmo0801
hpt (lmo0838)
srtA (lmo0929)
lplA1 (lmo0931)
dltA (lmo0974)
aut (lmo1076)
tagB (lmo1088)
codY (lmo1280)
inlK (lmo1290)
oatA (lmo1291)
lisK (lmo1378)
lisR (lmo1377)
mdrM (lmo1617)
lap (lmo1634)
lapB (lmo1666)
virS (lmo1741)
virR (lmo1745)
purQ (lmo1769)
inlC (lmo1786)
stp (lmo1821)
fbpA (lmo1829)
lspA (lmo1844)
lpeA (lmo1847)
fur (lmo1956)
inlL (LMON_RS10535)
lmo2026
bsh (lmo2067)
srtB (lmo2181)
svpA (lmo2185)
oppA (lmo2196)
prsA2 (lmo2219)
inlP (lmo2470)
pdeE (lmo2491)
lgt (lmo2482)
gtcA (lmo2549)
ami (lmo2558)
inlJ (lmo2821)

(A)


Serogroup
IVb
IVb-v1
IIb
IIa
IIc
L
Others/Unknown
Source
Human
Food-associated
Others/Unknown
Continent
North America
Europe
Others/Unknown
SL (cgMLST)
CC (MLST)
Serogroup
LIPI-1
LIPI-3
LIPI-4
gltA
glpB
tagB
gtcA
inlA
inlB
inlC
inlE
inlF
inlG
inlH
inlJ
inlK
aut
aut_IVb
bsh
brcABC
ermC
Tn6188::ermC
ermE
SSI-1
SSI-2
comK
Source
Continent
SL1
SL4
SL2
SL6
Lineage I
SL5
SL9
SL7
SL8
SL155
SL12 1
II
III
IV
0
25
50
75
100
similarity cgMLST (%)

(B)

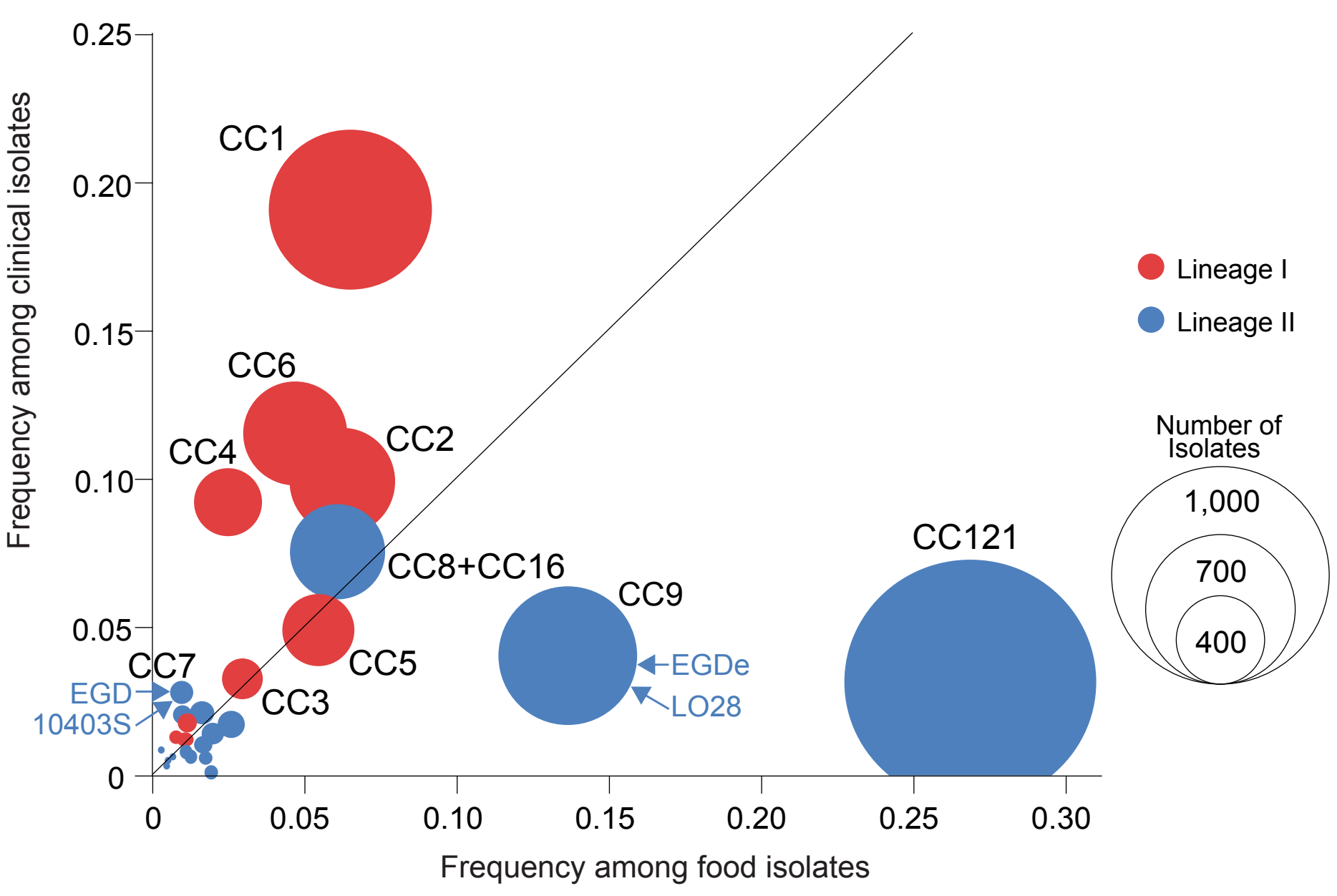
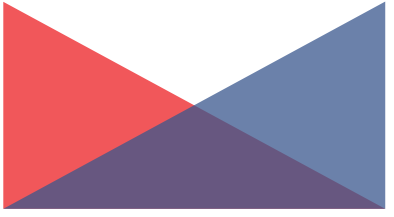

Frequency among clinical isolates
0.25
0.20
0.15
0.10
0.05
0
CC1
CC6
CC2
CC4
CC8+CC16
CC9
CC121
CC5
CC7
CC3
EGD
10403S
EGDe
LO28
0
0.05
0.10
0.15
0.20
0.25
0.30
Frequency among food isolates
Lineage I
Lineage II
Number of Isolates
1,000
700
400

Figure 3



Virulence

Immunodeficiency





Listeriosis depends on microbial and host factors

Host-association

Virulence
Active PrfA
Active LIPIs
Functional InlA
Fecal shedding
Transmission

Saprophytism

Loss of virulence
Inactive PrfA
Loss of LIPI-1 regulation
Truncation of InlA
Biofilm formation
Resistance to antiseptics