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Comparative Genomics Suggests Differences Related to Resistance and Virulence between Food-Isolated *Listeria monocytogenes* Serotypes 1/2a and 4b

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Abstract

Among the four lineages described for *Listeria monocytogenes* (I, II, III, and IV), lineages I and II harbor the serotypes most closely related to listeriosis in humans. Serotypes 1/2b and 4b are associated with the majority of listeriosis outbreaks, and serotype 1/2a is frequently involved in food and processing plant contamination. As such, the present study utilizes phylogenetic analysis for the aim of determining genomic differences between two *L. monocytogenes* strains isolated in southern Brazil (serotypes 1/2a and 4b) and known reference strains (*L. monocytogenes* EGD-e and *L. monocytogenes* Scott A). The Illumina Miseq platform was used to perform genomic sequencing, and cluster analysis of orthologous groups facilitated the investigation of similarities and differences between the two serotypes studied. In line with previous research, the studied strains of serotypes 1/2a and 4b presented different proteins related to resistance and virulence that may represent adaptations to several conditions during its evolution.

Keywords: *Defense Mechanisms; Listeriosis; Next Generation Sequencing; Orthologs; Resistance; Virulence*

Abbreviations

*Lm*55G: *Listeria monocytogenes* Serotype 1/2a; *Lm*47G: *Listeria monocytogenes* Serotype 4b; *Lm*EGD-e: *Listeria monocytogenes* EGD-e; *Lm*ScottA: *Listeria monocytogenes* Scotty A

Introduction

Listeria monocytogenes is a psychrotrophic, foodborne pathogen capable of growing during the storage and processing of refrigerated foods; this pathogen can cause listeriosis, a disease presenting a high mortality rate through specific risk-groups. Moreover, this microorganism is considered a ubiquitous bacterium, often surviving in conditions that are averse to bacterial development, which subsequently contributes to its widespread occurrence in nature and presence in food processing environments. Notably, approximately 99% of listeriosis cases (i.e. nearly all human cases) occur due to the ingestion of contaminated food, primarily containing serotype 1/2a, 1/2b, and 4b microorganisms.

Four evolutionary lineages have been described for *L. monocytogenes* (I, II, III, and IV). Lineages I and II harbor the serotypes most causative of listeriosis in humans, such as serotypes 1/2b and 4b (lineage I) and serotype 1/2a belonging (lineage II). Most listeriosis outbreaks are associated with lineage I serotypes, while lineage II appears to be more frequently involved in food contamination. Besides the frequent contamination of ready-to-eat foods, meats, and cheeses, this microorganism can be found in other several raw products that also require low temperature storage, particularly vegetables, milk, and fish. The primary reservoir of *Listeria* are the ruminants that facilitate its presence in milk, and consequently on dairy products.

Comparative genomic analyses are useful for identifying differences that may explain the ability of microorganisms to cause infections and the mechanisms involved in virulence processes, which vary from one strain to another. The verification of mutations becomes critical for understanding microorganism behavior; for example, Bécavin., *et al.* [1] demonstrated genomic differences between important reference strains of *L. monocytogenes*, highlighting a mutation in the transcription factor PrfA of *L. monocytogenes* EGD that induces overexpression of virulence genes - indicating a greater capacity for the microorganism to invade cell cultures. Additionally, Pasquali., *et al.* [2] studied the persistence of *L. monocytogenes* ST121 and ST14 repeatedly isolated within one year of sampling in a rabbit meat processing plant. Using a genomic approach, it was suggested that ST121 primarily includes strains resistant to sanitizers, which may partially explain the high detection frequency of this subtype in food processing plants; however, ST14 includes biofilm producer strains, which suggests that ST14 might occasionally contaminate harborage sites where sanitizing procedures are difficult to perform.

Therefore, the present study aims to analyze genomic differences of two *L. monocytogenes* strains (serotypes 1/2a and 4b) isolated from cheeses in southern Brazil against other widely used reference strains (*L. monocytogenes* EGD-e (serotype 1/2a) and *L. monocytogenes* Scott A (serotype 4b).

Materials and Methods

Bacterial strains

Two *L. monocytogenes* strains from serotypes 1/2a and 4b were used, named *Lm*55G and *Lm*47G respectively, both isolated from cheese samples by the National Agricultural Laboratory of Rio Grande do Sul State (LANAGRO/RS), from the Ministry of Agriculture, Livestock and Food Supply (MAPA/Brazil), and ELISA serotyped at the Oswaldo Cruz Institute (State of Rio de Janeiro - RJ, Brazil). For bacterial cells enrichment, Brain Heart Infusion (BHI; HiMedia, Mumbai, Maharashtra, India) broth was used, with subsequent culture on selective media plates containing Listeria Enrichment Broth (LEB; Thermo Fisher Scientific, Waltham, MA, USA) and bacteriological agar (HiMedia) at 37ºC [3].

Genome sequencing and assembly

Isolation of genomic DNA occurred through the PureLink™ Genomic DNA kit (Thermo Fisher Scientific), according to the manufacturer's instructions for Gram Positive bacterial cells, and parameters such as extraction yield and quality of genomic DNA were measured by spectrophotometry at 260 and 280 nm (Ultrospec 3100 Pro; Amersham Biosciences, Little Chalfont, UK). Before library preparation with the Nextera DNA Library Preparation kit 24 samples (Illumina, San Diego, CA, USA), genomic DNA samples were quantified on a fluorimeter (Qubit® 2.0; Thermo Fisher Scientific). Genome sequencing was performed on MiSeq Gene and Small Genome Sequencer (Illumina) equipment using the MiSeq Reagent kit v3 150 cycles (Illumina). One paired-end library of 76 bp reads was generated from each strain, and the quality of sequencing was analyzed using *FastQC* software [4], while *Trim Galore!* software (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to simultaneously remove Illumina adapter sequences and trim ends of reads of low quality.

The filtered reads from *Lm*55G and *Lm*47G were assembled *de novo* with *ABYSS* software [5] using kmer=31 and kmer=65 parameter, respectively. The obtained scaffolds were orientated using alignment script NUCmer from *MUMmer3* package [6], using *L. monocytogenes* EGD-e (*Lm*EGD-e) as reference genome.

Genome annotation

*Lm*55G and *Lm*47G genomes were annotated with Rapid Annotation using Subsystems Technology (*RAST*) [7] and Gene Ontology (GO) were assigned using *Blast2GO* [8]. Whole gene sequences in *RAST* were doubled checked using *BLASTx* against *Lm*EGD-e as reference genome.

Phylogenetic analysis

The listeriolysin O (*hly*) gene sequences retrieved from *Lm*55G and *Lm*47G were compared against the same gene present in other available complete genomes (Supplementary Material 1). The sequences were aligned and manually edited using *BioEdit v. 7.2.5* software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). Phylogenetic tree was constructed by the neighbor-joining method using *MEGA version 6* [9]. The robustness of the tree topology was evaluated by bootstrap analysis based in 1000 replicates.

Cluster analysis of orthologs

OrthoMCL v.2.0.5 program was used to identify the orthologous groups [10]. The algorithm pairs sequences using an all-versus-all *BLAST* and then clusters the pairs to orthologues groups using the Markov Clustering Algorithm (*MCL*) program. Aminoacid sequences obtained from *RAST* annotation (*Lm*55G and *Lm*47G) and from NCBI (*Lm*EGD-e and *L. monocytogenes* Scott A; *Lm*ScottA) and all standard parameters (a percent match cutoff =50 and E-value exponent cutoff=10^{-5}) were used in *OrthoMCL*. The graphical representation of relationships between the different strains was generated using *VennDiagram* package from R [11].

Data access

The whole genome shotgun projects for *Lm*55G and *Lm*47G have been deposited in *GenBank* [12].

Results and Discussion

Genome sequencing and gene annotation

Genome assembly statistics for the two studied *L. monocytogenes* strains are presented in table 1. Notably, *Lm*55G presented a higher number of scaffolds and mapping-rate percentage, with a media contig size of 439.603 compared to 258.151 for *Lm*47G. In contrast, *Lm*47G demonstrated greater values for genome coverage and number of reads. Furthermore, *Lm*47G exhibited an increased number of genes (3.026) compared to *Lm*55G, *Lm*EDG-e and *Lm*ScottA, which presented 2.873, 2.867, and 2.969 genes, respectively (Table 2). For all four strains, the total coding region was approximately 89% of the full draft genome sequence.

	Lm55G	Lm47G
Total scaffolds	30	24
Total bases in scaffolds	3.026.679	3.036.711
Scaffolds N50 (bases)	439.603	258.151
Scaffolds max (bases)	865.036	483.808
Total reads	4.780.984x2	9.107.687x2
Fold-coverage	123.4	227
Mapping-rate (%)	94	90
G+C content (%)	37.8	37.9

Table 1: *Listeria monocytogenes 55G and Listeria monocytogenes 47G genome assembly statistics.*

	***Lm*55G**	***Lm*47G**	***Lm*EGD-e**	***Lm*ScottA**
CDS	2.873	3.026	2.867	2.969
tRNA	24	65	67	67
rRNA	3	6	18	18
CDS (length)	2.593.612	2.728.182	2.629.341	2.688.456
CDS (%)	89.77%	89.47%	89.29%	88.96%
Total sequence length	2.889.017	3.049.032	2.944.528	3.021.822

Table 2: *Draft genome annotation statistics.*
CDS: Coding Sequence.

Based on the phylogenetic tree obtained with the *hly* gene (Figure 1), *Lm*EGD-e and *Lm*ScottA were selected for comparative genetic analysis against *Lm*55G and *Lm*47G, respectively.

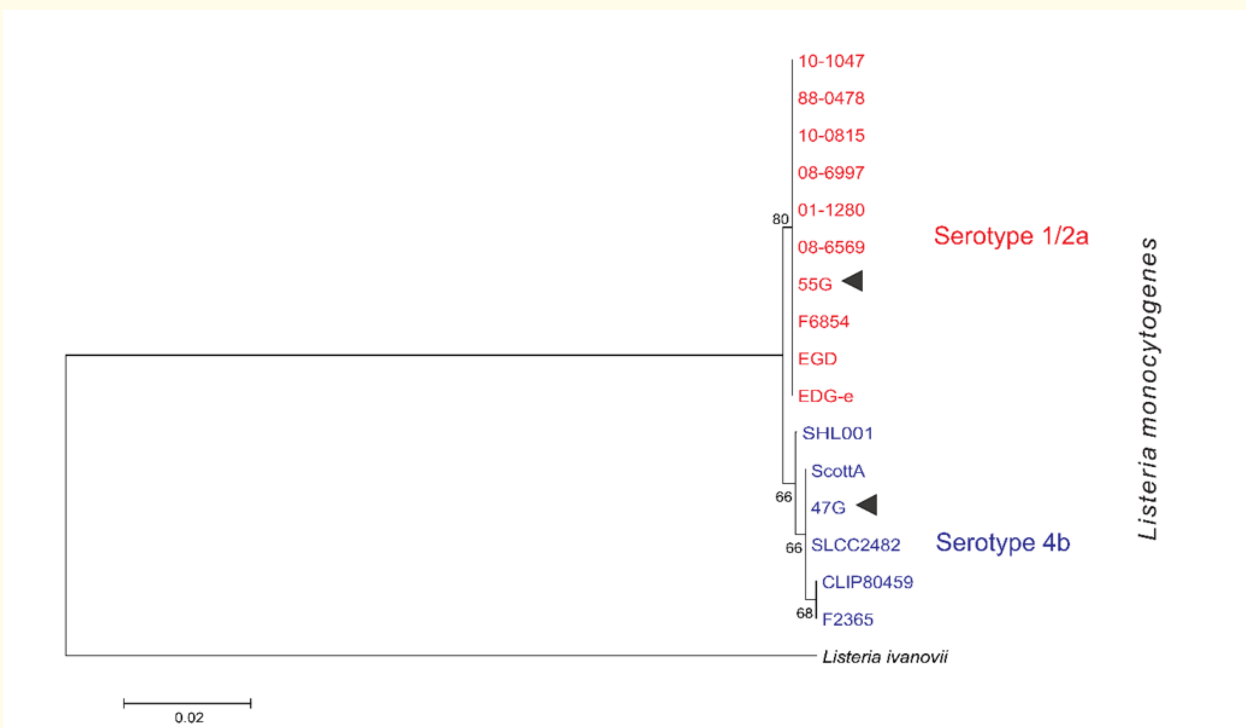

Figure 1: *Neighbor-joining phylogenetic tree based on hly gene sequences showing the relationships of Listeria monocytogenes serovars. Bootstrap values (>60%) based on 1,000 resamplings are shown above the nodes. Bar, 0.02 substitution per nucleotide position. Listeria ivanovii was used as the outgroup. Graphical representation obtained with MEGA software version 6.*

Orthologous genes between serotypes 1/2a and 4b

OrthoMCL was used to investigate orthologous groups between *Lm*55G, *Lm*47G, *Lm*EGD-e, and *Lm*ScottA, and a total of 2.587 genes were shared between the four *L. monocytogenes* strains based on a 10 aa *cutoff* (Figure 2). A total of 153 orthologous genes were present in serotype 1/2a strains, *Lm*55G, and *Lm*EGD-e (Supplementary Material 2), while a higher number of orthologous genes were identified in serotype 4b strains, *Lm*47G, and *Lm*ScottA (equal to 162 genes) (Supplementary Material 3).

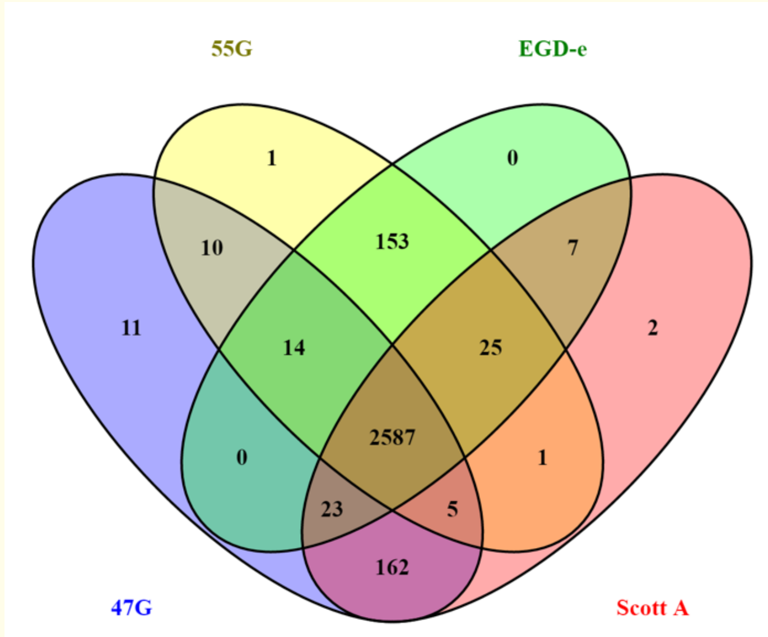

Figure 2: *Venn Diagram as a graphical representation of relationships between the different strains of Listeria monocytogenes studied. Graphical representation obtained with VennDiagram package from R.*

Among the 153 orthologous genes between *Lm*55G and *Lm*EGD-e, it is important to note the presence of some genes and operons involved in microorganism abilities, such as cell invasion, virulence, and environmental adaptation. As in the serotype 1/2a strains studied, *Lm*47G and *Lm*ScottA also shared a large proportion of genes relevant to microorganism pathogenesis and resistance, which is vital to its survival under several conditions.

*Lm*EGD-e and *Lm*ScottA were considered as important references that have been widely used in *L. monocytogenes* research [1,13], which justifies its use in comparative genomics performed in the present work.

Orthologous genes of serotype 1/2a strains

Late genes (*lmo2278-2301*) from *Lm*EGD-e comprise genes coding structural, assembly and DNA packaging proteins, and also proteins required for host cell lysis such as LysA (*lmo2278*), a peptidoglycan hydrolase encoded by genomes of lytic phages that lyse the host cell and release the phage progeny. Fundamental for the adherence and invasion of host cells, the internalins are of great importance to the microorganism pathogenicity. In the present study, a group of surface protein-encoding genes (*lmo0801*, *lmo1289*, *lmo2027*, *lmo0171*, and *lmo0262-inlG*) were shared only between 1/2a serovar strains *Lm*EDG-e and *Lm*55G. According to Garmyn., *et al.* [14], a virulence genes cluster and several internalins of *Lm*EGD-e (*inlA*, *inlB*, *inlC*, *inlE*, *inlG*, *lmo0801*, *lmo1289*, and *lmo2027*) presented higher transcription levels at 37°C in comparison to results at 25°C, indicating that temperature is involved in the regulation of *L. monocytogenes* pathogenicity.

Regarding *lmo0171*, the results of the present study are congruent with Zhang and Knabel [15], as this internalin, like *inlJ*, *inlL*, *lmo0327*, *lmo0732*, *lmo2026*, and *lmo2396*, contains a mucin-binding domain (MucBP) that is found in bacterial peptidoglycan-bound proteins, and plays a role in microorganism adhesion to surfaces. Also, disruption of *lmo0171* resulted in important cell morphology alterations alongside the decreased ability of *L. monocytogenes* to invade eukaryotic cell lines as well as diminished adhesion efficiency, which demonstrates the role of *lmo0171* role during the early stages of listerial infection. Some LPXTG proteins are also involved with the microorganism attachment to surfaces and its virulence. Examples are Lmo2576, suggested to have an ability to interact with collagen that provides a general advantage to the bacteria in pathogenesis, and Lmo0435 (BapL), indicated as involved in attachment to abiotic surfaces of polystyrene and stainless steel that contributes to *L. monocytogenes* surface adherence during biofilm formation [16]. Important to note that the region between *lmo0430* and *lmo0435* corresponds to where the *L. monocytogenes inlAB* operon involved in cell invasion and *Listeria* pathogenicity is located. Additionally, the orthologous gene *lmo2550* is also involved with adhesion and biofilm formation ability of *L. monocytogenes*. According to Zhang., *et al*. [17], *lmo2055* was not detected in *L. monocytogenes* serovar 4b strains.

Genes present in *Lm*EGD-e (*lmo0444*, *lmo0445*, *lmo0446 [pva]*, *lmo0447 [gadD1]*, and *lmo0448 [gadT1]*) comprise a region known as stress survival islet 1 (SSI-1), which is responsible for *Lm*EGD-e growth at low pH and high salt concentrations, and its ability to survive and grow in model food systems. It has been demonstrated that the islet genes are internally regulated by the σ^B-dependent gene *lmo0445*, suggesting that this regulator may contribute to the capacity of *L. monocytogenes* to respond and adapt to the various environmental conditions encountered in either foods or hosts [18]. Both *lmo0445*, a candidate regulator of virulence genes, and *lmo0446*, which encodes bile acid hydrolases in response to acid stress, were only shared between 1/2a serovar strains *Lm*55G and *Lm*EGD-e. The two-component systems (TCS) aid bacteria in adapting to a wide variety of stress conditions, and Chan., *et al.* [19] demonstrated the contribution of several TCS including Lmo1060 (*lmo1061/lmo1060*) and of alternative σ factors σ^C and σ^H to *L. monocytogenes* cold adaptation and shock response. In accordance, Pöntinen., *et al.* [20] attempted to elucidate the role of TCS histidine kinases (HK) on *L. monocytogenes* growth at low temperatures, indicating that several HK-encoding genes, including *lmo1061*, exhibited increased expression levels following cold shock from 37°C to 5°C. The *sigC* gene cluster (*lmo0421-lmo0423*) was also only shared between *Lm*EGD-e and *Lm*55G in the present study and this result is congruent with those of den Bakker., *et al.* [21] and Zhang., *et al* [17]. This operon, strongly induced by temperature increase, is composed by *lmo0421*, that encodes the RodA protein involved in cell wall elongation; *lmo0422* (*lstR*, for the lineage-specific thermal regulator), the actual thermal resistance regulator or effector involved in heat shock response of the microorganism; and *lmo0423* (*sigC*, which codes for σ^C) that provides a mechanism for temperature-dependent transcription of *lmo0422.*

Some orthologous genes between serotype 1/2a strains are mainly involved with sugar metabolism, such as genes cluster (*lmo0734-lmo0739* and *lmo1968-lmo1974)* and the operon *bvrABC*. Among these genes, the present work only identified *lmo0738* (phosphotransferase system - PTS - IIABC) as not shared between the 1/2a strains studied. This *bvr* locus (*bvrABC*), involved in β-glucoside metabolism, encodes an anti-terminator of the BglG family (*lmo2788; bvrA*), which acts as a β-glucosidase repressor; a β-glucoside-specific enzyme II permease component of the phosphoenolpyruvate-sugar phosphotransferase system (*lmo2787*, *bvrB*); and a putative ADP-ribosylglycohydrolase (*lmo2786; bvrC*). Its presence is only shown in *L. monocytogenes*, being this operon absent in other *Listeria* species, and the *bvrABC* locus appears to be lacking from the genome of serotype 4b strains. It encodes a β-glucoside-specific sensor that mediates virulence gene repression upon detection of cellobiose and salicin. This indicates that *bvr* was the first sensory system found in *L. monocytogenes* involved in the environmental regulation of virulence genes [22].

ATP binding and hydrolysis energy are used to transport substances such as sugars, ions, amino acids, and peptides across cellular membranes by ATP-binding cassette (ABC) transporters. *Lm*55G and *Lm*EGD-e shared an ABC transporter complex (*lmo1062 and lmo1063*) and sugar ABC transporters *lmo0768 (ugpB)*, *lmo0767 (ugpE)* and *lmo0766 (ugpA),* components of the *ugpBCEA* operon. The *sn*-glycerol-3-phosphate transport system includes *ugpB*, *ugpC*, *ugpE*, and *ugpA* genes and is also considered a putative virulence-associated determinant of microorganisms. Other important orthologous proteins that are located closely and involved in components transport are the *tatAC* gene cluster *(lmo0361 to lmo0362)*, coding for the twin arginine secretion and transport apparatus, and the *fepCAB* operon (*lmo0365* to *lmo0367),* involved in iron transport. Ledala., *et al.* [23] determined that both those gene clusters are under the regulation of a ferric uptake regulator (Fur) of intracellular iron in bacteria, which presence and iron acquisition ability is of critical importance to bacteria survival in the host as well as *L. monocytogenes* virulence. Also, it seems that an iron-dependent peroxidase encoded by *lmo0367* may play a role in oxidative stress response.

Orthologous genes of serotype 4b strains

Considered an environment contaminant of water and soils, arsenic enters the biosphere primarily through geological formations and anthropomorphic sources including arsenic-containing fungicides as well as pesticides and herbicide application. Both prokaryotic and eukaryotic cells possess defense mechanisms against this metal organized in *ars* operons. In this work, *Lm*47G and *Lm*ScottA demonstrated the presence of *arsABD* genes (ArsA - LMOSA_RS41825 and LMOSA_RS41840; ArsB - LMOSA_RS41845 and LMOSA_RS41850; ArsD - LMOSA_RS41820 and LMOSA_RS41830) and according to Lee., *et al.* [24] the *Lm*ScottA genome genes related to arsenic resistance are part of an arsenic resistance cassette within a 35-kb chromosomal region termed the *Listeria* genomic island 2 (LGI2). In this way, LMOSA_RS40775 in *Lm*ScottA encodes AcrR, a TetR/AcrR family transcriptional regulator that recognizes toxic compounds or stress signals. TetR proteins constitute a well-known family of transcriptional repressors that are recognized in the regulation of several genes for drug efflux systems. Moreover, the role of *acrR* in microorganism antibiotic resistance has been demonstrated by the overexpression of efflux pumps [25].

Related with the microorganism resistance, LMOSA_RS41810 encodes an extracytoplasmic function (ECF) sigma factor (σ^{70}-ECF) in *Lm*ScottA, which was only shared with *Lm*47G in the present study. Bacterial sigma factors are essential components of RNA polymerase that determine promoter selectivity. The sigma factor 70 family (σ^{70}) coordinates the transcriptional activities in various stress responses by activation of genes involved in the microorganism resistance to antimicrobial compounds and other processes that affect the cell envelope, including iron uptake, cell wall maintenance, and motility [26]. It is suggested that in bacterial pathogens such as *L. monocytogenes*, σ^{70} family members serve as links between bacterial abilities to respond to changes imposed by the host environment and, subsequently, disease. About DNA methyltransferases (MTases), in bacteria they play a critical role through the regulation of gene expression to help such microorganisms cope with environmental changes in nutrient availability, temperature, pH, and osmolarity, beyond their involvement with pathogenesis. MTases in bacteria have been classified into two primary groups, one of which is associated with restriction modification systems (R-M systems, classified into types I through IV), and other with orphan or solitary MTases that comprise DNA adenine MTase (Dam), cell cycle-regulated MTase (CcrM), and DNA cytosine MTase (Dcm) [27]. In the present work, Dcm (LMOSA_RS30790; LMOSA_RS32225), Type I restriction-modification system subunit M (LMOSA_RS32145), and Type I restriction endonuclease subunit R (LMOSA_RS32155) were shared only between *Lm*ScottA and *Lm*47G. Also, a restriction endonuclease Mrr (encoded by LMOSA_RS32160 in *Lm*ScottA) and a class I S-adenosyl-methionine (SAM)-dependent DNA methyltransferase (LMOSA_RS35940, which englobes a region related to the Type II restriction/modification system DNA methylase subunit YeeA) were only present in the serovar 4b strains studied. Mrr is part of the type IV R-M system and is activated by mild high hydrostatic pressure application and specifically targets methylated DNA, while YeeA has a proposed function in *Lactococcus lactis* energy metabolism as a maltose hydrolase [28].

Both LMOSA_RS29470 and LMOSA_RS30655 possess a region identified as the SseB protein C-terminal domain and SseB protein N-terminal domain, respectively. *Salmonella*-derived SseB is a protective antigen encoded by the *Salmonella* pathogenicity island 2 (SPI-2) that promotes bacterial survival and is crucial for microorganism virulence due to its involvement in the infection process [29]. Still about pathogenicity, GntR family members belong to the helix-turn-helix group of bacterial transcriptional regulators and are involved in the regulation of many different biological processes, including primary metabolism, motility, development, antibiotic production and resistance, plasmid transfer, and virulence. In the present work, the transcriptional regulator GntR (LMOSA_RS32570) was only shared between serovar 4b strains *Lm*ScottA and *Lm*47G. In *L. monocytogenes*, GntR was identified within the CodY regulon, indicating a hierarchy in the regulation of the *codY* gene, which controls the expression of both metabolic and virulence genes in Gram-positive bacteria [30]. Equaly involved with virulence, LMOSA_RS35160 refers to a phospholipid carrier-dependent glycosyltransferase. It has a region identified as the protein ArnT, a member of the PMT family belonging to Gram-negative bacteria such as *E. coli* and *Salmonella enterica* serovar Typhimurium, which are involved in the immune escape. This protein masks the lipid A molecule *in vivo* by its glycosylation in order to avoid recognition by Toll-like receptor 4 (TLR4), that detects lipopolysaccharides (LPS) of Gram-negative bacteria. The lipid A molecule, as part of the LPS, is known to play a major role in septicemia. As LPS modifications contribute to pathogenicity and innate immunity evasion, ArnT can be considered a virulence factor.

Important genes related to transport and metabolism were also only shared between *Lm*ScottA and *Lm*47G. An example is the MurR/RpiR family transcriptional regulator (RpiR DNA-binding transcriptional regulator), encoded by LMOSA_RS38835 in *Lm*ScottA. The RpiR family is composed of regulatory proteins that control carbohydrate metabolism in both Gram-negative and Gram-positive bacteria, such as ribose metabolism, regulation of *N*-acetylmuramic acid catabolism in *E. coli*, maltose transport and metabolism in *B. subtilis,* and the pentose phosphate pathway in *S. aureus*. It is also strongly suggested that RpiR mediates one of the several mechanisms by which virulence and metabolism are coupled [31]. About nitrogen compounds, LMOSA_RS41855 of *Lm*ScottA has a region identified as the protein NirB, which is a NAD (P) H-nitrite reductase important to energy production and conversion. Some bacteria can metabolize nitrate into nitrite as well as nitrite into nitric oxide, and nitric oxide production has been demonstrated to increase antibiotic resistance of some Gram-positive bacteria such as *Bacillus anthracis* and *S. aureus* [32]. Related to the catabolism of carbohydrates, LMOSA_RS31890 refers to the ABC-type maltose transport system MalG, which was shared only between *Lm*ScottA and *Lm*47G in the present work. Some firmicutes use a single ABC transporter for the efficient uptake of maltose and maltodextrins in which the maltose-specific ABC transporter system is composed of MalE, MalF, and MalG [33,34]. Interestingly, in some species of *Streptococcus* maltodextrin use is linked to virulence factor production. Also, LMOSA_RS32750 of *Lm*ScottA has two regions identified as "uncharacterized conserved protein RhaS", which is identified as a rhamnose metabolism regulator in *E. coli*. RhaS belongs to the AraC/XylS family, which is found in a wide range of both Gram-positive and Gram-negative bacteria species, and is related to diverse cellular functions, including carbon metabolism, various stress responses (e.g. antibiotic biosynthesis), and pathogenesis [35].

Several other genes shared between 4b strains are involved with cell motility, adherence to surfaces and biofilm formation. The *ccmA* gene (ABC-type multidrug transport system/ATPase component encoded by LMOSA_RS30425 in *Lm*ScottA) is the first gene of an eight-protein-encoded operon involved in cytochrome *c* maturation and heme delivery though heme uptake from the environment [36]. Some studies indicate a relationship between *ccmA* and microorganism's adhesion, biofilm and lipopolysaccharides biosynthesis, as also cell motility, morphology and division. According to Hay., *et al.* [37] CcmA has been shown to affect cell shape in *Proteus mirabilis*, which can be influenced by nutrient access, cell division and segregation, attachment to surfaces, and active motility. LMOSA_RS31900 of *Lm*ScottA encodes a sucrose phosphorylase (sucrose_gtfA), and GtfA is part of a group of glycosyltransferases, the major virulence factors in dental caries caused by *Streptococcus mutans.* In *S. mutans*, synthesis of glucan (component of dental plaque) is stimulated by the GtfA enzyme, being the adherence of this bacterium to smooth surfaces related to sucrose-dependent cell-cell aggregation. In *Streptococcus pneumoniae*, GtfA and GtfB form an oligosaccharyltransferase complex to *O*-GlcNAcylate the pneumococcal SRR protein adhesin (PsrP) involved in infection and pathogenesis [38]. Also, a ParA family protein is encoded by LMOSA_RS40750 in *Lm*ScottA, which has a region that comprises the cellulose biosynthesis protein BcsQ, commonly related to cell motility and biofilm formation. Le Quére and Ghigo [39] demonstrated that BcsQ (also named YhjQ or WssA) displays a polar localization, while cell-to-cell adhesion is initiated through cellulose production at the BcsQ-labeled pole.

The bacteria cell wall is frequently remodeled, degraded, and rebuilt during bacterial growth and cell division. Therefore, peptidoglycan recycling is an important process in which bacteria import cell wall degradation products and incorporate them back into either peptidoglycan biosynthesis or basic metabolic pathways [40]. Litzinger., *et al.* [41] introduced the evidence of a muropeptide catabolic pathway for cell wall recovery in a Gram-positive organism distinct from that performed by *E. coli* and other Gram-negative bacteria. In *B. subtilis*, one identified pathway used for the recovery of *N*-acetylglucosamine (GlcNAc)-*N*-acetylmuramic acid (MurNAc) peptides (muropeptides) derived from the peptidoglycan of the cell wall was encoded by a cluster of six genes. The first three genes are orthologs of *E. coli* and involved in *N*-acetylmuramic acid dissimilation that encodes a MurNAc-6-phosphate etherase (MurQ, encoded by LMOSA_RS38830 in *Lm*ScottA), a MurNAc-6-phosphate-specific transcriptional regulator (MurR), and a MurNAc-specific phosphotransferase system (MurP). Borisova., *et al.* [42] indicated that three Gram-positive model organisms, including *S. aureus* and *B. subtilis*, are able to recycle the sugar MurNAc of their peptidoglycan during growth in rich medium via the presence of the MurNAc-6-phosphate (MurNAc-6P) etherase (MurQ) enzyme. Moreover, quantification of MurNAc-6P in Δ*murQ* cells of *S. aureus* and *B. subtilis* indicated that recycling predominantly occurs during the transition to stationary phase, providing the benefit of long-term survival in these microorganisms. Another protein that acts in the peptidoglycan of bacteria cell walls is *N*-acetylmuramoyl-L-alanine amidase CwlA (encoded by LMOSA_RS30970 in *Lm*ScottA), a lytic amidase from *B. subtilis*. This cell wall hydrolase cuts linkages in the peptidoglycan, cleaving the peptide side chain from the glycan backbone and acting as an autolysin, which is important for growth and transverse processes of cell fission related to microorganism pathogenesis.

Conclusions

Several genes related to resistance and virulence of *L. monocytogenes*, including its capacity for biofilm formation, were shared between the serovars studied. As comented previously, lineage I (including serovar 4b) is the most related to listeriosis in humans, while lineage II (including serovar 1/2a) appears to be more involved in food contamination, being frequently isolated from food processing plants. Based on these references, it can be suggested that serotype 4b may present more virulence factors, while serotype 1/2a probably developed a major number of mechanisms involved with adherence to surfaces and biofilm formation.

According to Martinez., *et al.* [43], that investigated *L. monocytogenes* pathogenesis in the *Galleria mellonella* insect model, no significant differences in virulence were observed among the serotypes 1/2a, 1/2b and 4b tested. Albeit, Nightingale., *et al.* [44] demonstrated that strains with reduced pathogenicity displaying truncated and non-functional virulence factors such as internalin are commonly isolated from food, where *L. monocytogenes* serotype 1/2a is commonly found. Maury., *et al.* [45] identified full-length InlA, *Listeria* pathogenicity island 3 (LIPI-3, or listeriolysin S cluster), and gene clusters responsible for teichoic acid biosynthesis in serotype 4b strains strongly associated with its infectious potential. As well, Lee., *et al*. [22] show that arsenic resistance is encountered primarily in serotype 4b clones, considered to have enhanced virulence. As described in the present work, only *Lm*ScottA and *Lm*47G (serotype 4b strains tested) shared an *ars* operon for defense mechanisms against arsenic.

Di Bonaventura., *et al.* [46] experiments demonstrated no significant difference in biofilm formation between lineages I and II of *L. monocytogenes*. Otherwise, according to Folsom., *et al.* [47] *L. monocytogenes* serotype 4b strains had their capacity for biofilm formation reduced when the level of nutrients in the medium used in the experiments decreased, whereas the same was not observed for strains of serotype 1/2a. In the study performed by Pan., *et al.* [48] serotype 1/2a strains formed denser biofilms than serotype 4b strains under a variety of conditions. A similar result was obtained through Combrouse., *et al.* [49] experiments, as *L. monocytogenes* lineage II strains produced significantly more biofilm than lineage I strains in different conditions of temperature and media. Likewise, Borucki., *et al.* [50] showed higher levels of biofilm production by lineage II strains. These informations corroborate that lineage II *L. monocytogenes* strains, including serotype 1/2a, has a good potencial for biofilm formation. Important to note that in several epidemic cases of listeriosis, the persistence strains of *L. monocytogenes* were reported in an industrial environment, being related to this resistance its capacity to form biofilms.

Finally, using comparative genomics, differences in the two major serotypes of *L. monocytogenes* species associated with human outbreaks were demonstrated in the present study. These differences represent adaptations to different conditions that occurred during evolution.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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