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MLVA typing of *Listeria monocytogenes* strains isolated from brain samples of sheep, goat and cattle, and comparison with strains of human, food and environmental origin

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ABSTRACT

Listeria monocytogenes is among the most important food-borne pathogens and is well adapted to persistence in the environment. Several studies evidence the existence of differences in the pathogenicity between *L. monocytogenes* strains. In order to get an insight into the genetic relatedness of *L. monocytogenes* strains originating from different sources, we applied a multi locus variable number tandem repeat analysis (MLVA) to subtype 197 *L. monocytogenes* strains isolated from humans, ruminants, food and environment. Allelic profile-based comparisons clustered *L. monocytogenes* strains into 8 clonal complexes. Clonal complexes A and C emerged to be the main ones, containing 67 and 58 strains out of 197, respectively. The clonal complex A consisted of 95.5% (64/67) of strains isolated from brain samples independently of the species. Moreover, strains isolated from bovine brain samples were restricted to the clonal complex A. In contrast, the majority of food and environmental strains were clustered in the clonal complex C, and none of food and environmental strains were located in clonal complex A. The association between clonal complexes and the origin of the strain appeared to be statistically highly significant. Subsequently, strains of the two main clonal complexes (A and C) obtained by MLVA were analyzed by PCR for the presence of 12 virulence-associated genes (*prfA*, *actA*, *inlA*, *inlB*, *inlC*, *inlE*, *inlF*, *inlG*, *inlJ*, *inlC2*, *auto*, and *vip*), which revealed significant differences in the *actA* and *inlJ* genes between clinical and non-clinical strains. The presence of different *actA* and *inlJ* alleles suggested that particular alleles might be very important for *L. monocytogenes* virulence.

INTRODUCTION

Listeria monocytogenes, a Gram-positive bacterium, is an important food-borne pathogen for humans as well as for several animal species [13]. *L. monocytogenes* is widespread in the environment due to its eurythermal and osmotolerant characteristics, and the ability to grow at a wide range of pH [2, 13], which makes the control of infection by this pathogen difficult. Unlike infection with other food-borne pathogen, listeriosis causes high case-fatality rates of 20–30% [8, 12, 15]. After ingestion of contaminated food, which is the main cause of infection, *L. monocytogenes* is able to colonize the gastrointestinal tract of several mammalian species, thus allowing systemic spread of the bacterium and causing invasive disease. The diverse clinical manifestations, such as meningitis, encephalitis, septicemia, abortion, perinatal infections, and gastroenteritis, reflects *L. monocytogenes* ability to cross three tight barriers in the host [4, 11, 15]. Clinical outcome of *Listeria* infection depends on three major variables: the number of bacteria ingested, the pathogenic properties of the strain, and the immunological status of the host [34]. Domesticated ruminants probably play a key role in the maintenance of *Listeria* spp. in the rural environment via a continuous fecal-oral enrichment cycle [34]. Clinically asymptomatic sheep, goats and cattle often shed *L. monocytogenes* in their faeces [36], thus contaminating pastures or vegetables, surface waters and milk [12]. This is particularly indicative by the fact that unpasteurized dairy products represent the major problem of human *L. monocytogenes* infections [12]. Among ruminants, sheep seem to be particularly susceptible to *L. monocytogenes* infection that causes mainly central nervous system (CNS) lesions, while cattle seem to be less affected [5, 23].

L. monocytogenes is a facultative intracellular pathogen that can invade and multiply in both professional phagocytic and in particular in non-phagocytic cells [7, 13, 14, 29, 34]. In order to achieve this, *L. monocytogenes* tightly controls the expression of a broad range of virulence

genes to assure the production of the necessary virulence effectors at the specific sites along the process of invasion and infection of the host tissue. Major virulence determinants, such as the internalin A (InlA) and InlB (internalization), the cholesterol dependent pore forming bacterial toxin listeriolysin (LLO), phospholipases PlcA and PlcB (escape from the host vacuole), the actin-based motility surface protein ActA (triggers bacterial intracellular motility via interactions with the Arp2/3 complex, a multiprotein complex that nucleates and cross-links actin filaments into arrays), or the master regulator of virulence PrfA are conserved in virulent and in less virulent strains of *L. monocytogenes*. Hence, the presence of these genes alone is not enough to explain the differences in virulence of any one particular strain [7, 21, 34]. Several cell wall-associated proteins are involved in *L. monocytogenes* virulence, such as internalin-like proteins InlE, InlF, InlG, InlH, which are not involved in invasion process, but are important for the colonization of host tissues *in vivo* [17, 34], as well as autolysin Auto, which is necessary but not sufficient for the entry [10, 11], and invasion-mediating protein Vip, which is required for invasion of several cell lines [10]. The recently discovered internalin-like protein InlJ is required for full virulence of *L. monocytogenes in vivo* [10]. Moreover, internalin C (InlC) and InlC2 may play an important role in specific host-associated niche [33]. Particular to *L. monocytogenes* is that it can induce its entry into a broad range of non-phagocytic mammalian cells [25]. For this purpose, the bacterial surface-anchored protein InlA interacts with its cellular receptor E-cadherin to promote invasion into polarized intestinal epithelial cells, while InlB interacts with the hepatocyte growth factor (Met) to trigger entry into hepathocytes and non-polazized cells [16, 19, 20, 30]. Furthermore, interactions between host cell receptors and InlA or InlB are species-specific [17]. The concerted action between InlA, InlB, and InlJ (the latter being specifically expressed during infection *in vivo*) is required for the invasion of certain tissues under *in vivo* condition, such as placenta [9, 18]. Interestingly, *L. monocytogenes* does not express the *inlJ* gene when

infecting mammalian cell lines *in vitro*, but seems to play a role in colonization of liver and spleen, and particularly in causing bacteraemia in blood [28].

For epidemiological surveillance and outbreak investigations, serotyping is the standard method and a first step for subtyping strains of *L. monocytogenes* [3]. Clinical isolates from human mostly belong to serotype 4b and 1/2b, while in samples from food, which are not outbreak-related, the most frequent serotypes are 1/2a and 1/2c [1, 12, 22, 24]. More recently, a multi locus variable number tandem repeat analysis (MLVA) method has been developed for *L. monocytogenes* genotyping that allowed clustering strains of *L. monocytogenes* with a strong epidemiological concordance [32]. The method is based on the analysis of the number of small repeated units present in eight different variable number tandem repeat (VNTR) loci that encode mostly for extracellular invasion-associated proteins [32]. Hence, the method not only clusters strains purely according to their genetic relatedness, but being based on potential virulence markers, is also indirectly biased towards the ecological niche and the pathogenic potential of the strains [6]. In the present study we have used this method to cluster 197 strains of *L. monocytogenes* isolated from two main sources: *i*) clinical isolates from brains of small ruminants and cattle with listeric encephalitis and a few human isolates; and *ii*) strains isolated from food and the environment, collected from two geographically and climatically different countries. Subsequently, strains of the two main clonal complexes obtained by MLVA were analyzed for the presence and structure of 12 virulence-associated genes, which revealed significant differences in the *actA* and *inlJ* genes between clinical and non-clinical strains.

MATERIAL AND METHODS

Bacterial strains

A collection of 197 *L. monocytogenes* strains were investigated in this study (Table 1). The collection contained 109 strains from brains of ruminants with listeric encephalitis, 4 strains from placenta of bovine abortions, 10 strains from human neonatal infections, 10 from human cerebrospinal fluid (CSF), 40 strains from non-outbreak-related food from Switzerland and Greece, 10 strains isolated from the environment, and 14 strains of other mostly unknown origin. Strains from human and from Swiss-food, as well as environmental strains and strains of unknown origin were obtained from the Swiss National Centre for Listeriosis (Centre Hospitalier Universitaire Vaudois CHUV, Lausanne, Switzerland). Other strains were identified using the VITEK® 2 compact system (bioMérieux, Geneva, Switzerland) according to the operating instructions and subsequently confirmed by an in-house-validated real-time PCR using specific primers and probe for the detection of the *hly* gene of *L. monocytogenes* [26].

Strain growth conditions and lysates

Strains were grown on Triptic Soy agar (TSA) containing 5% sheep blood (Oxoid, Basingstoke, UK). For genotyping analysis, material from 5 colonies was lysed in 450 µl lysate buffer containing 0.1 M Tris–HCl, pH 8.5, 0.05% Tween 20, and 0.24 mg/ml of proteinase K for 1 hour at 60°C followed by inactivation of the proteinase K for 15 min at 97°C. Lysed samples were kept at –20°C until further analysis.

Multi Locus Variable number tandem repeat Analysis (MLVA)

Eight reference loci (Lm-2, Lm-3, Lm-8, Lm-10, Lm-11, Lm-15, Lm-23, Lm-32) and eight corresponding primer pairs, previously described by Sperry et al. [32], were used to perform typing of *L. monocytogenes* strains by MLVA. All selected loci have relatively small repeat units of 6 base pairs (bp) to 15 bp and are able to distinguish serotypes 1/2a, 1/2b, and 4b [32]. All selected primers were manufactured by Microsynth AG (Balgach, Switzerland). Specificity of all primers was verified using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The eight loci were tested in single polymerase chain reactions (PCR) before combining them in a multiplex PCR assay. Multiplexed PCR, described by Sperry et al. [32], was modified and consisted of four multiplexed PCRs with two primer loci in each reaction: R-1 (Lm-2, Lm-8), R-2 (Lm-10, Lm-11), R3 (Lm-3, Lm-23) and R4 (Lm-15, Lm-32). PCR products were amplified using the Qiagen® Multiplex PCR Kit (Qiagen AG, Basel, Switzerland) as per manufacturer's instructions. Amplification was performed using DNA thermal cycler Gene Amp 9600 (Applied Biosystems, Foster City, CA). The cycling conditions were as follows: initial denaturation step at 95°C for 15 min, followed by 35 cycles of 3-step cycling at 94°C for 30 s, 52°C for 90 s and 72°C for 90 s, and one cycle of final extension at 72°C for 10 min. DNA fragments were separated on Agilent 2100 bioanalyzer using the Agilent DNA 1000 kit (Agilent Technologies, Waldbronn, Germany) according to manufacturer's standard protocol. Agilent 2100 bioanalyzer allowed the estimation of DNA fragment length without the use of fluorescent primers. DNA 1000 kit sizing accuracy was ±10% (for ladder as samples). The final concentration of DNA in the samples did not exceed 50 ng/µl.

A Microsoft Excel file was created to calculate the number of repeats in each locus according to Sperry et al. [32]. Briefly, the fragment size with no tandem repeats (a) was subtracted from the amplicon size (x), then divided by the repeat unit length (b) ((x-a)/b). A categorical

minimum spanning tree (MST) was created using BioNumerics software (version 5.1; Applied Math, Sint-Martens-Latem, Belgium) from MLVA data.

Analysis of twelve virulence-associated genes by PCR and DNA-sequencing

Five randomly chosen strains from each of the two main complexes A and C, as obtained by MLVA, were analyzed by PCR for the presence of twelve virulence-associated genes (*prfA*, *actA*, *inlA*, *inlB*, *inlC*, *inlE*, *inlF*, *inlG*, *inlJ*, *inlC2*, *auto*, and *vip*) using primers listed in Table 2. Subsequently, the *actA* and *inlJ* genes, which showed size variations, were PCR-amplified from all strains of this study and analyzed for their length. PCR amplification was performed with 35 cycles and an annealing temperature of 54 °C.

DNA sequencing of PCR fragments was performed with an ABI PRISM™ 3100 Genetic Analyzer (Aplied Biosystems) and BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instruction. Editing of sequences was done using the Sequencher 4.6 sofware (GeneCodes, Ann Arbor, MI).

Statistical analysis

Contingency tables were used to measure the statistical significance between clonal complexes, ActA protein, InlJ protein, and the origin of the strain using the Fisher's exact test (www.graphad.com). For this statistical analysis we included 183 strains out of 197.

RESULTS

Allelic profile-based comparisons from MLVA clustered *L. monocytogenes* strains into 8 clonal complexes, which were arbitrary named A, B, C, D, E, F, G, and H. Clonal complexes A and C emerged to be the main ones, containing 67 and 58 strains out of 197, respectively (Table 3 and Figure 1). Complex A (n = 67) consisted of 95.5% (n = 64) *L. monocytogenes* strains isolated from neuropathological samples (n = 119) (Figure 1a). These 67 samples included 44.7% ovine, 34.3% bovine, 13.4% caprine, and 6% human strains. The only 3 strains in complex A that were other than isolates from neuropathologies were from bovine placenta (n = 1) and human neonatal infections (n = 2). No strain from food and environment clustered in complex A. Particularly noticeable is the fact that bovine strains isolated from brain samples appeared to have restricted diversity, clustering in complex A (22/27). Moreover, and in agreement with Sperry et al. [32], MLVA was able to differentiate serotypes (1/2a, 1/2b, and 4b) for those strains of which serotype information was available. Strains with serotype 4b were found in complex A and those with serotype 1/2a in complex C (Table 1). Most food and environmental strains, 70% (35/50), clustered in clonal complex C independently of their geographical origin (Figure 1b). The remaining strains in complex C were isolated from brains of sheep, goat, cattle and human, human neonatal infections, and bovine placenta (Figure 1, Table 1). Clonal complex B, that is linked to complex A, consisted also mainly of strains from neuropathological samples (11/14) from ovine, bovine and human origin. The remaining 3 strains were from 2 human neonatal infections and 1 food strain of serotype 4b.

The possible association between MLVA clonal complexes and virulence-associated genes was analyzed by PCR. Initially, we screened the presence of 12 virulence-associated genes (*prfA*, *inlA*, *inlB*, *inlC*, *inlE*, *inlF*, *inlG*, *inlJ*, *inlC2*, *actA*, *auto*, and *vip*) in 5 strains of each of

the two most representative clonal complexes, A and C. Results of PCR targeting these genes, except for *actA* and *inlJ*, revealed no differences for strains of these two clonal complexes. In contrast, PCR amplifications of the *actA* and *inlJ* genes revealed two and three different fragment sizes, respectively. In order to characterize *actA* and *inlJ* variants in detail, the respective PCR fragments from strains L86/2007, L11/2007 (for *actA*) and strains L11/2007, L41//2007 and CHUV 206/2005 (for *inlJ*) were sequenced. Two alleles of *actA* were denominated as *actA3* (962 bp) and *actA4* (1067 bp) according to previous observations [35]. DNA fragment alignment showed the absence of 105-bp fragment in allele *actA3*, which in allele *actA4* encodes for a repeated 29-amino acid stretch followed by a 6-amino acid proline-rich region (PRR) (Figure 2a). Comparison of ActA3 and ActA4 with the corresponding region of *L. monocytogenes* type strain EGDe revealed that this latter contains allele *actA4*, containing 4 PRRs. Three alleles of *inlJ* of length 780 bp, 570 bp and 357 bp were denominated as *inlJ1*, *inlJ2* and *inlJ3*, respectively. DNA fragment alignment of the 3 various *inlJ* alleles and comparison with *L. monocytogenes* type strain EGDe is shown in Figure 2b. Allele *inl2* has the same length as *inlJ* in strain EGDe, but leads to 13 different amino acids. Allele *inlJ1* is significantly larger and *inlJ3* is significantly shorter than *inlJ* in strain EGDe. The 3 alleles of *inlJ* differ mainly in the number of mucine binding domains (MucBP): InlJ1 has 5 MucBP, InlJ2 has 4, and InlJ3 has 2 complete MucBP.

PCR analysis of *actA* and *inlJ* genes of all strains used in this study revealed that these differences in fragment sizes correlate to the MLVA clonal complexes (Figure 3). Generally, the shorter allele *actA3* is found in the two clonal complexes A and B that contain clinical strains, while the larger allele *actA4* is found in complex C that contains the strains isolated from food and environmental samples. Resulting data concerning *actA* and *inlJ* alleles were added to the MST and revealed that strains with *actA3* and *inlJ1* are mainly located in complex A, which consists mainly of strains isolated from brain samples (64/67). Sixty-six

strains of clonal complex A out of 67 (98.5 %) harbored *actA3* and *inlJ1*. In contrast, 50/58 (86.2%) strains of clonal complex C had allele *actA4* and 52/58 (89.6%) allele *inlJ2*. Most notable, all 27 *L. monocytogenes* strains isolated from bovine brain samples carried the shorter allele *actA3*, while strains isolated from bovine placenta (n = 4) had alleles *actA4* (3/4) or *actA3* (1/4) (Table1).

DISCUSSION

Pathogenicity of *L. monocytogenes* requires the activity of a particular broad range of virulence factors that are accurately regulated at the transcriptional and/or translational level in order to enable the pathogen to cross the relevant tissue cells and to propagate in certain tissues before reaching the final targets [4, 19, 25, 28]. Beside the constitution of the virulence apparatus of the pathogen, the particular conditions of the host's susceptibility to the pathogen also are determinant for the severity of the infection. In this respect, it is noticeable that among ruminants, cattle seems to be most resistant to invasion by *L. monocytogenes*, while small ruminants, such as goats and sheep, are much more susceptible, a fact that has been proven both epidemically as well as experimentally [5]. In our molecular epidemiological study by typing 197 *L. monocytogenes* strains using MLVA, strains were clustered in 2 major (A and C) and 6 minor (B, D, E, F, G, and H) complexes, and in various single locus variants (SLVs). Complex C and its linked SLVs mostly grouped non-disease related strains from food and environmental samples originating from geographically different places. In contrast, the other major complex A and the linked minor complex B were representative for pathogenic strains from human and animal neuropathological samples that were isolated from individuals with neurological symptoms and/or histopathological lesions characteristic for *L. monocytogenes* infection. Strains isolated from brains of cattle from various regions in Switzerland revealed to be particularly tightly clustered in complex A. Since cattle generally are less susceptible to listeriosis, we speculated that this complex would contain particularly virulent strains of *L. monocytogenes*. In contrast, animals such as goats and sheep, which are more susceptible to listeriosis, and humans seem to be infected to a certain extent also with strains from the complex C that seems to contain less virulent strains.

The presence of different *actA* and *inlJ* alleles suggests that particular alleles might be very important for *L. monocytogenes* virulence. Interestingly, Wiedman et al. [35] observed *L. monocytogenes* strains that have three PRR in ActA. It is worth to note at this point, that PRR stimulates *L. monocytogenes* movement by providing profilin-actin complex that fuel the actin polymerization via the vasodilator-stimulated phosphoprotein (VASP) [16]. Moreover, Wiedman et al. [35] showed a unique signature sequence FPLMP in the third ActA PRR, in contrast to FPP(I/M)P. This unique ActA signature sequence has linked for the first time a distinct virulence gene allele to this bacterial clone responsible for the majority of the human listeriosis outbreaks. Undeniably, this allele can be used to rapidly detect this clone. Likewise, this ActA sequence type with three PRRs has been linked to increased virulence and was assumed to specifically cause listerial encephalitis in ruminants [35]. Interestingly, our results correlate with these findings (Figure 2a). Moreover, Smith et al. [31] evaluated the ActA protein function and demonstrated a quantitative role for the PRRs in the localization of VASP and profilin at the bacteria/tail interface and in the rate of actin-based motility. In our study, almost all *L. monocytogenes* strains (66/67) with ActA3 (3 PRRs) are located in clonal complex A (Figure 3a) that holds the majority of the strains isolated from brain samples. Concerning *inlJ* gene, Sabet et al. [27, 28] reported that the *inlJ* is only expressed *in vivo* and deletion mutants are significantly attenuated in virulence after intravenous infection of mice or oral inoculation of transgenic mice expressing E-cadherin. In our study, we showed that InlJ2 and InlJ3 are mostly located in clonal complex C (Figure 3b), and not in A, where 66 strains out of 67 (98.5%) have InlJ1. This indicates that InlJ1 is associated with the origin of the strain, precisely with brain.

A subsequent analysis of the virulence-associated genes of *L. monocytogenes* strains revealed that particular alleles of the genes encoding the actin-based motility surface protein ActA and InlJ are strongly associated to the various MLVA complexes. Fisher's exact test revealed a

statistically highly significant association between clinical strains with the *actA3* and *inlJ1* alleles isolated from human and animals ($P < 0.0001$), and also a strong association between strains with alleles *actA4* and *inlJ2* or *actA4* and *inlJ3* with environmental and food origin ($P < 0.0001$). Interestingly, allele *actA3* seemed to be determining neural diseases, in particular listeric encephalitis in cattle, because the majority (66/67) of *L. monocytogenes* strains with this allele are located in clonal complex A (Figure 3a), which harbors the majority of the strains isolated from brain samples.

In summary, the results of this study showed that the MLVA method developed by Sperry et al. [32] for *L. monocytogenes* genotyping represents an excellent alternative for epidemiological studies of listeriosis, and most likely for the detection of epidemiologically linked cases. We consider that the MLVA method used in this study has a great advantage over other typing methods due to the fact that this MLVA includes loci encoding putative membrane associated proteins, which are probably under high selective pressure during infection. Moreover, we speculate that *L. monocytogenes* strains having *actA3* and *inlJ1* alleles are significantly enhanced in virulence. However, this can only be evaluated by construction of knockout strains and *in vivo* experiments.

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FIGURE LEGENDS

Figure 1. Minimum spanning tree analysis of 197 *L. monocytogenes* strains based on 8 genetic markers. Closed circles represent strains. Circle size is proportional to the number of strains. Color zones surround the circles that belong to the same complex. (**a**) Red circles indicate strains isolated from brain samples. (b) Red circles indicate strains isolated from food and environment.

Figure 2. (a) Alignment of partial predicted ActA amino acid sequences for the two different *actA* alleles. PRR regions are marked in grey. The dashes indicate the 35-amino acids missing in ActA3, consisting of the 29 amino acids from the long repeat (underlined) and one 6-amino acids long PPR region. L86 (ActA4) and L11 (ActA3) predicted amino acid sequences are compared to strain EGDe ActA protein with accession number CAA42407. The amino acid variations in ActA3 are indicated with black background. (b) Alignment of partial predicted InlJ amino acid sequences for the 3 different *inlJ* alleles. L11 (InlJ1), L41 (InlJ2) and CHUV 206/2005 (InlJ3) predicted amino acid sequences are compared to strain EGDe InlJ protein with accession number NP_466343. The dashes indicate the missing amino acids. The amino acid variations are indicated with black background.

Figure 3. Minimum spanning tree analysis of 197 *L. monocytogenes* strains based on 8 genetic markers. Closed circles represent strains. Circle size is proportional to the number of strains. Color zones surround the circles that belong to the same complex. (a) Yellow circles indicate strains with allele *actA3*, red circles – strains with allele *actA4*. (b) Red circles indicate strains with allele *inlJ1*, yellow and blue circles – strains with allele *inlJ2* and *inlJ3* respectively.

TABLE 1. L. monocytogenes strain information

Strain information				MLVA locus copy number[b]								Allele[c]		Complex
Strain[a] (serotype)	Origin	Origin type	Country of origin	Lm-2	Lm-8	Lm-10	Lm-11	Lm-3	Lm-23	Lm-15	Lm-32	*actA*	*inlJ*	
L135/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	3	18	3	1	A
L138/2007	Bovine	Brain	Switzerland	19	3	3	5	8	21	2	14	3	2	C
L140/2007	Bovine	Brain	Switzerland	17	3	3	5	2	17	2	18	3	1	A
L142/2007	Bovine	Brain	Switzerland	17	3	2	5	2	18	3	18	3	1	A
L143/2007	Bovine	Brain	Switzerland	19	3	3	-2	7	21	2	14	3	2	SLV[f]
L146/2007	Bovine	Brain	Switzerland	17	3	2	5	2	18	2	18	3	1	A
L81/2007	Bovine	Brain	Switzerland	17	3	3	5	2	18	3	18	3	1	A
L101/2007	Bovine	Brain	Switzerland	17	4	3	5	2	18	3	19	3	1	A
L102/2007	Bovine	Brain	Switzerland	17	4	3	5	2	18	3	19	3	1	A
L103/2007	Bovine	Brain	Switzerland	17	3	2	5	2	23	3	18	3	1	A
L104/2007	Bovine	Brain	Switzerland	17	3	3	5	2	18	3	18	3	1	A
L105/2007	Bovine	Brain	Switzerland	17	3	2	5	2	18	2	18	3	1	A
L108/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	2	18	3	1	A
L110/2007	Bovine	Brain	Switzerland	17	3	3	5	2	18	3	18	3	1	A
L113/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	2	18	3	1	A
L114/2007	Bovine	Brain	Switzerland	16	2	4	4	2	14	4	17	3	2	B
L115/2007	Bovine	Brain	Switzerland	16	3	3	5	2	18	-2	20	3	1	A
L117/2007	Bovine	Brain	Switzerland	16	3	3	5	1	18	2	18	3	1	A
L118/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	3	18	3	1	A
L119/2007	Bovine	Brain	Switzerland	17	3	3	5	2	18	3	18	3	1	A
L120/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	2	18	3	1	A
L122/2007	Bovine	Brain	Switzerland	17	2	4	4	3	14	3	24	3	2	SLV[f]
L123/2007	Bovine	Brain	Switzerland	17	3	3	5	2	17	3	18	3	1	A
L133/2007	Bovine	Brain	Switzerland	17	3	3	5	2	17	2	18	3	1	A
L134/2007	Bovine	Brain	Switzerland	17	3	2	5	2	17	4	23	3	1	A
L161/2008	Bovine	Brain	Switzerland	17	2	4	4	3	17	5	17	3	1	B
O/D562/09	Bovine	Brain	Switzerland	17	3	2	5	1	17	2	18	3	1	A
A156	Bovine	Placenta	Switzerland	19	3	3	4	8	22	2	14	4	2	C
O/D1387/06	Bovine	Placenta	Switzerland	19	3	3	4	2	15	2	14	4	2	C
O/D36/08	Bovine	Placenta	Switzerland	18	4	3	5	2	18	3	19	3	1	A
O/D1171/06	Bovine	Placenta	Switzerland	21	3	3	4	2	11	6	15	4	2	SLV[f]
L11/2007	Goat	Brain	Switzerland	18	4	3	5	3	18	3	19	3	1	A
L9/2007	Goat	Brain	Switzerland	18	4	2	5	2	18	3	19	3	1	A

L21/2007	Goat	Brain	Switzerland	17	2	3	5	3	17	3	19	3	1	A
L32/2007	Goat	Brain	Switzerland	17	3	2	5	2	18	3	19	3	1	A
L33/2007	Goat	Brain	Switzerland	17	3	3	5	2	18	3	19	3	1	A
L40/2007	Goat	Brain	Switzerland	20	3	3	4	3	13	1	15	4	2	C
L45/2007	Goat	Brain	Switzerland	18	3	3	5	9	20	1	12	4	2	SLV[f]
L46/2007	Goat	Brain	Switzerland	21	3	3	3	1	13	2	12	4	2	SLV[f]
L47/2007	Goat	Brain	Switzerland	19	2	3	1	2	16	1	13	4	2	SLV[f]
L48/2007	Goat	Brain	Switzerland	19	3	3	5	14	21	2	14	4	2	C
L49/2007	Goat	Brain	Switzerland	18	3	3	5	2	12	2	14	4	2	C
L50/2007	Goat	Brain	Switzerland	21	3	3	4	1	22	1	14	4	2	SLV[f]
L52/2007	Goat	Brain	Switzerland	22	3	3	4	7	19	2	14	4	2	C
L126/2007	Goat	Brain	Switzerland	17	3	2	5	2	20	-2	15	4	2	SLV[f]
L127/2007	Goat	Brain	Switzerland	17	2	3	4	5	21	4	14	4	2	C
L129/2007	Goat	Brain	Switzerland	17	3	2	5	2	17	2	18	4	1	A
L84/2007	Goat	Brain	Switzerland	18	3	3	5	4	18	0	14	4	1	H
L87/2007	Goat	Brain	Switzerland	18	2	3	5	3	18	1	14	4	1	H
L95/2007	Goat	Brain	Switzerland	18	2	3	4	10	14	2	14	4	3	C
L96/2007	Goat	Brain	Switzerland	16	3	3	5	1	15	2	18	4	2	A
L130/2007	Goat	Brain	Switzerland	17	3	3	5	2	17	3	18	3	1	A
L148/2008	Goat	Brain	Switzerland	17	3	3	5	2	17	3	19	3	1	A
L131/2007	Goat	Brain	Switzerland	24	3	2	4	3	13	3	14	4	2	SLV[f]
L132/2007	Goat	Brain	Switzerland	22	3	3	4	6	20	2	15	4	2	SLV[f]
L2/2007	Sheep	Brain	Switzerland	18	3	3	5	2	11	3	19	3	1	A
L3/2007	Sheep	Brain	Switzerland	18	3	2	5	2	18	3	18	3	1	A
L4/2007	Sheep	Brain	Switzerland	18	4	6	5	2	18	3	19	3	1	SLV[f]
L5/2007	Sheep	Brain	Switzerland	19	3	3	5	9	21	2	14	3	2	C
L10/2007	Sheep	Brain	Switzerland	23	3	3	5	10	22	2	15	4	2	SLV[f]
L13/2007	Sheep	Brain	Switzerland	18	3	5	4	3	15	5	18	3	2	B
L18/2007	Sheep	Brain	Switzerland	15	3	3	5	2	21	3	18	4	1	F
L22/2007	Sheep	Brain	Switzerland	22	3	3	5	5	22	2	15	4	2	SLV[f]
L26/2007	Sheep	Brain	Switzerland	17	3	3	3	2	17	3	19	3	1	A
L27/2007	Sheep	Brain	Switzerland	18	4	3	5	1	18	3	19	3	1	A
L28/2007	Sheep	Brain	Switzerland	18	4	2	5	2	18	3	19	3	1	A
L34/2007	Sheep	Brain	Switzerland	22	3	2	5	2	18	2	18	3	1	A
L35/2007	Sheep	Brain	Switzerland	19	3	3	5	4	12	1	14	4	2	H
L36/2007	Sheep	Brain	Switzerland	19	3	3	1	2	16	2	15	4	2	SLV[f]
L38/2007	Sheep	Brain	Switzerland	17	2	3	4	5	18	3	14	3	1	C
L39/2007	Sheep	Brain	Switzerland	17	3	2	5	2	18	2	18	3	1	A
L41/2007	Sheep	Brain	Switzerland	16	2	11	4	3	14	5	18	3	2	SLV[f]

L42/2007	Sheep	Brain	Switzerland	28	3	4	4	3	15	4	17	3	2	B
L43/2007	Sheep	Brain	Switzerland	18	3	3	4	2	19	2	15	4	3	C
L44/2007	Sheep	Brain	Switzerland	19	2	3	4	1	18	1	15	4	3	C
L51/2007	Sheep	Brain	Switzerland	16	2	3	4	1	21	2	14	4	2	C
L54/2007	Sheep	Brain	Switzerland	20	3	3	5	6	23	2	14	4	2	SLV[f]
L55/2007	Sheep	Brain	Switzerland	18	4	3	5	3	17	2	18	3	1	A
L56/2007	Sheep	Brain	Switzerland	9	3	4	5	2	17	2	18	3	1	A
L57/2007	Sheep	Brain	Switzerland	16	3	3	5	1	18	2	18	3	1	A
L58/2007	Sheep	Brain	Switzerland	17	3	3	5	2	17	2	18	3	1	A
L59/2007	Sheep	Brain	Switzerland	16	3	3	5	2	18	2	18	3	1	A
L60/2007	Sheep	Brain	Switzerland	16	3	3	5	1	18	2	18	3	1	A
L61/2007	Sheep	Brain	Switzerland	19	2	3	5	4	22	2	14	4	2	C
L62/2007	Sheep	Brain	Switzerland	18	3	3	5	2	18	2	18	3	1	A
L63/2007	Sheep	Brain	Switzerland	16	3	3	5	1	18	2	18	3	1	A
L64/2007	Sheep	Brain	Switzerland	18	3	3	5	2	18	3	19	3	1	A
L66/2007	Sheep	Brain	Switzerland	17	3	3	5	2	18	3	19	3	1	A
L67/2007	Sheep	Brain	Switzerland	17	3	3	5	2	18	3	19	3	1	A
L68/2007	Sheep	Brain	Switzerland	18	4	3	5	3	18	3	19	3	1	A
L69/2007	Sheep	Brain	Switzerland	15	3	3	5	3	18	3	19	3	1	A
L70/2007	Sheep	Brain	Switzerland	18	4	3	5	3	18	3	19	3	1	A
L71/2007	Sheep	Brain	Switzerland	18	4	3	5	2	17	3	19	3	1	A
L73/2007	Sheep	Brain	Switzerland	15	2	4	4	4	14	5	18	3	2	B
L74/2007	Sheep	Brain	Switzerland	19	3	3	4	4	16	1	14	4	2	SLV[f]
L76/2007	Sheep	Brain	Switzerland	30	3	3	4	6	12	2	14	4	2	SLV[f]
L77/2007	Sheep	Brain	Switzerland	17	3	5	4	3	15	5	18	3	3	B
L78/2007	Sheep	Brain	Switzerland	15	2	2	4	4	18	3	17	3	2	SLV[f]
O/D115/04	Sheep	Brain	Switzerland	20	3	3	4	11	21	2	14	4	2	C
L82/2007	Sheep	Brain	Switzerland	17	3	3	5	2	17	3	18	3	1	A
L83/2007	Sheep	Brain	Switzerland	17	4	3	5	2	18	3	19	3	1	A
L85/2007	Sheep	Brain	Switzerland	17	4	3	5	2	17	2	18	3	1	A
L86/2007	Sheep	Brain	Switzerland	23	3	3	5	2	11	2	14	4	2	SLV[f]
L88/2007	Sheep	Brain	Switzerland	17	4	3	5	2	18	2	19	3	1	A
L90/2007	Sheep	Brain	Switzerland	17	3	2	5	2	18	2	18	3	1	A
L92/2007	Sheep	Brain	Switzerland	17	3	3	5	2	18	3	18	3	1	A
L93/2007	Sheep	Brain	Switzerland	17	3	3	5	2	17	5	23	3	1	A
L97/2007	Sheep	Brain	Switzerland	19	3	3	4	7	22	2	14	4	2	C
L147/2008	Sheep	Brain	Switzerland	17	3	2	5	2	18	3	18	3	1	A
L157/2008	Sheep	Brain	Switzerland	17	3	4	4	3	15	5	17	3	2	B
L162/2008	Sheep	Brain	Switzerland	17	2	4	4	3	15	5	17	3	2	B
L23/2007	Sheep	Brain	Switzerland	14	3	3	4	3	18	2	14	3	2	C
O/D1463/07	Alpaca	Endocardium	Switzerland	19	3	3	4	2	15	2	17	4	2	SLV[f]

L145/2007	Deer	Brain	Switzerland	17	4	3	5	2	18	-2	20	3	1	A
CHUV 186/2006 (4b)	Human	CSF[e]	Switzerland	17	2	4	4	3	14	4	17	3	2	B
CHUV 016/2007 (4b)	Human	CSF[e]	Switzerland	17	2	4	4	3	14	4	17	3	2	B
CHUV 162/2006 (4b)	Human	CSF[e]	Switzerland	17	2	4	4	3	14	4	17	4	2	B
CHUV 083/2006 (1/2b)	Human	CSF[e]	Switzerland	18	2	2	5	5	16	3	15	3	1	D
CHUV 072/2006 (4b)	Human	CSF[e]	Switzerland	14	2	3	5	2	22	2	17	4	1	F
CHUV 052/2006 (1/2a)	Human	CSF[e]	Switzerland	21	3	3	4	5	14	1	14	4	2	C
CHUV 037/2006 (4b)	Human	CSF[e]	Switzerland	18	4	3	5	2	18	2	18	3	1	A
CHUV 227/2005 (1/2a)	Human	CSF[e]	Switzerland	17	3	8	−2	5	15	1	14	nd[d]	2	SLV[f]
CHUV 212/2005 (4b)	Human	CSF[e]	Switzerland	18	4	2	5	3	18	3	19	3	1	A
CHUV 200/2005 (1/2a)	Human	CSF[e]	Switzerland	23	3	3	-2	3	14	0	13	4	2	SLV[f]
CHUV 009/2007 (1/2a)	Human	Neonatal inf.	Switzerland	18	3	3	-2	2	12	2	14	4	2	G
CHUV 176/2006 (4b)	Human	Neonatal inf.	Switzerland	17	3	2	3	3	15	5	18	3	2	B
CHUV 141/2006 (4b)	Human	Neonatal inf.	Switzerland	17	3	4	4	3	15	5	18	3	2	B
CHUV 128/2006 (1/2a)	Human	Neonatal inf.	Switzerland	21	3	3	-2	2	12	2	14	3	2	G
CHUV 253/2005 (4b)	Human	Neonatal inf.	Switzerland	17	4	3	6	2	17	3	19	3	1	A
CHUV 203/2005 (4b)	Human	Neonatal inf.	Switzerland	14	3	3	5	2	21	2	18	3	1	F
CHUV 092/2005 (1/2a)	Human	Neonatal inf.	Switzerland	19	3	3	4	8	19	2	14	4	2	C
CHUV 091/2005 (1/2a)	Human	Neonatal inf.	Switzerland	19	3	3	4	8	20	2	14	4	2	C
CHUV 053/2005 (1/2a)	Human	Neonatal inf.	Switzerland	17	2	2	5	2	12	1	14	4	2	C
CHUV 144/2006 (4b)	Human	Neonatal inf.	Switzerland	6	3	2	5	2	18	2	19	3	1	A
CHUV 174/2006 (1/2b)	Food		Switzerland	16	2	3	5	4	16	3	16	3	1	SLV[f]
CHUV 055/2007 (1/2a)	Food		Switzerland	21	3	3	4	2	12	2	14	4	2	C
CHUV 022/2007 (1/2a)	Food		Switzerland	22	3	3	4	9	13	2	17	4	2	SLV[f]
CHUV 019/2007 (1/2a)	Food		Switzerland	23	3	3	4	7	12	-2	14	4	2	SLV[f]
GL11	Food	Poultry	Greece	22	2	3	4	7	11	2	14	4	2	C
GL5	Food		Greece	21	3	3	2	6	22	2	14	4	2	C
GL7	Food	Poultry	Greece	19	3	3	4	7	12	2	14	4	2	C
CHUV 007/2007 (1/2a)	Food		Switzerland	17	3	3	4	2	16	3	17	4	2	SLV[f]
CHUV 005/2007 (4b)	Food		Switzerland	17	2	5	4	3	14	4	17	3	2	B
CHUV 181/2006 (1/2a)	Food		Switzerland	23	3	3	4	7	12	2	14	4	2	C
CHUV 179/2006 (1/2a)	Food		Switzerland	17	3	3	4	2	20	4	15	3	3	C
CHUV 178/2006 (1/2a)	Food		Switzerland	13	1	3	4	5	21	-2	14	4	2	SLV[f]
GL1	Food		Greece	12	2	2	4	-2	12	-2	14	4	2	SLV[f]
GL2	Food	Meet prod.	Greece	18	2	3	4	6	22	1	14	4	2	C
GL4	Food	Meet prod.	Greece	19	2	3	4	6	8	2	14	4	2	C
GL6	Food	Poultry	Greece	18	2	3	4	5	8	1	14	4	2	C
GL8	Food	Poultry	Greece	19	3	3	4	2	4	2	14	4	2	C
GL9	Food	Poultry	Greece	20	3	3	2	6	8	2	14	4	2	C

GL10	Food	Poultry	Greece	21	3	3	7	6	11	2	14	4	2	C
GL12	Food	Beef	Greece	23	2	2	3	7	2	2	14	4	2	C
GL13	Food	Beef	Greece	20	3	3	4	6	8	2	14	4	2	C
GL14	Food	Beef	Greece	22	3	2	4	6	2	1	15	4	2	C
GL15	Food	Beef	Greece	23	3	3	4	7	2	2	14	4	2	C
GL16	Food	Cheese	Greece	23	3	3	4	7	2	2	14	4	2	C
GL17	Food	Cheese	Greece	21	2	3	4	6	2	1	14	4	2	C
GL18	Food	Cheese	Greece	24	3	3	4	7	2	2	14	4	2	C
GL19	Food	Cheese	Greece	22	3	3	4	6	22	2	15	4	2	C
GL20	Food	Fish	Greece	22	3	3	4	6	21	2	14	4	2	C
GL21	Food	Fish	Greece	21	3	3	4	6	22	2	14	4	2	C
GL22	Food	Fish	Greece	24	3	3	4	7	11	2	14	4	2	C
GL23	Food	Fish	Greece	21	3	3	4	6	22	2	14	4	2	C
GL24	Food	Fish	Greece	25	3	3	4	7	11	2	14	4	2	C
GL25	Food	Fish	Greece	24	3	3	4	7	11	2	14	4	2	C
GL26	Food	Meet prod.	Greece	21	3	3	4	6	22	-2	15	4	2	C
GL28	Food	Meet prod.	Greece	25	3	3	4	7	11	2	14	4	2	C
GL29	Food	Meet prod.	Greece	25	3	3	4	7	11	2	14	4	2	C
GL30	Food	Meet prod.	Greece	21	3	4	5	6	21	2	14	4	2	C
GL31	Food	Meet prod.	Greece	19	3	4	5	6	22	2	15	4	3	SLV[f]
GL32	Food	Meet prod.	Greece	19	3	4	-2	6	22	2	15	4	2	SLV[f]
CHUV 031/2007 (1/2a)	Food		Switzerland	20	3	3	-2	2	12	-2	-2	4	2	G
CHUV 206/2005 (1/2a)	Environmental		Switzerland	18	2	3	4	2	21	4	15	3	3	C
CHUV 003/2005 (1/2a)	Environmental		Switzerland	20	3	3	4	6	23	2	14	4	2	C
CHUV 014/2005 (1/2b)	Environmental		Switzerland	17	2	3	5	4	16	3	15	3	1	D
CHUV 026/2005 (4b)	Environmental		Switzerland	13	3	2	5	2	22	3	18	4	1	F
CHUV 051/2005 (1/2b)	Environmental		Switzerland	17	3	3	2	3	16	1	16	3	1	SLV[f]
CHUV 062/2005 (1/2a)	Environmental		Switzerland	17	3	3	-2	2	12	1	14	4	2	SLV[f]
CHUV 153/2005 (1/2a)	Environmental		Switzerland	18	2	3	5	6	22	1	14	4	2	C
CHUV 157/2005 (1/2b)	Environmental		Switzerland	17	2	2	5	4	16	6	16	3	1	D
CHUV 188/2005 (1/2a)	Environmental		Switzerland	16	2	3	4	8	19	2	14	4	2	C
CHUV 031/2005 (1/2a)	Environmental		Switzerland	18	3	3	3	2	20	4	15	3	3	C
CHUV 4a (4a)	Unknown		Switzerland	13	3	3	4	-2	21	2	-2	3	2	SLV[f]
CHUV 1/2a (1/2a)	Unknown		Switzerland	18	3	4	4	2	12	2	15	4	2	SLV[f]
CHUV 1/2b (1/2b)	Unknown		Switzerland	18	2	4	6	7	15	5	18	4	1	SLV[f]
CHUV 1/2c (1/2c)	Unknown		Switzerland	22	2	4	5	7	11	2	15	4	2	SLV[f]
CHUV 3a (3a)	Unknown		Switzerland	17	2	4	-2	2	11	-2	15	4	2	SLV[f]
CHUV 3b (3b)	Unknown		Switzerland	17	2	4	4	6	10	4	19	3	1	SLV[f]
CHUV 3c (3c)	Unknown		Switzerland	22	2	4	5	7	12	2	15	4	2	C
CHUV 4ab (4ab)	Unknown		Switzerland	12	3	4	-2	-2	22	2	-2	nd[d]	2	SLV[f]

CHUV 4b (4b)	Unknown	Switzerland	17	3	4	6	2	17	3	19	3	1	E
CHUV 4c (4c)	Unknown	Switzerland	12	2	3	-2	-2	16	2	-2	4	2	SLV[f]
CHUV 4d (4d)	Unknown	Switzerland	16	2	2	4	2	12	5	18	4	1	SLV[f]
CHUV 4e (4e)	Unknown	Switzerland	10	4	4	6	2	17	3	19	3	1	E
CHUV 7 (7)	Unknown	Switzerland	18	3	3	5	7	15	5	18	4	1	SLV[f]

[a] CHUV, Centre Hospitalier Universitaire Vaudoise, Lausanne, Switzerland; L, O/D and A, Institute of Veterinary Bacteriology, ZOBA, Vetsuise Faculty, Bern, Switzerland; GL, Department of Medicine, Veterinary School, University of Thessaly, Greece.

[b] -2, null allele.

[c] *actA4* corresponds to 1067 bp, *actA3* - 962 bp, *inlJ1* - 780 bp, *inlJ2* - 570 bp, *inlJ3* - 357 bp.

[d] nd, DNA fragment not detected.

[e] CSF, cerebrospinal fluid.

[f] SLV, single locus variant.

TABLE 2. Primers used to amplify virulence-associated genes *actA*, *auto*, *prfA*, *inlA*, *inlB*, *inlC*, *inlD*, *inlE*, *inlF*, *inlG*, *inlJ*, *inlC2*, and *vip*

Gene	Forward primer (5' - 3)	Reverse primer (5' - 3)
actaA	GTTGATAAAAGTGCAGGGTTAATTG	GCAATTAGTTTTCCTTCTTCAATGC
actaA	TTTCCTTGTTCTAAAAAGGTTGTATTAGC	GTGGAAAGTCCGAAGCATTTACC
auto	AGAAGTGAATTAGGGAAGGCCC	AGTAGATCCAGCAGTGATTTTACC
prfA	ATGAACGCTCAAGCAGAAGAATTC	TTAATTTAATTTTCCCCAAGTAGCA
inlA	TAACAAAGCTTCAAAGATTATTTTTCTATAATAAC	TCAGTCAATAAATTCCCAGCTTCC
inlA	AAGCTGGGAATTTATTGACTGAACC	TGAATTATAAGGGTCATAAGCGTTCATT
inlB	TGGTTTTCGGACTATATCTAGCTTTT	TAAAATCGTTTCCGGACATATAATCC
inlC	TGCTAACATATAAGTATACAAAGGGACA	AACTGTTCCATCAAATATAGCCTCA
inlD	AACTCTGGCTGTAGTAATGGCAAT	CGCCAGTTTTCGCATCATAC
inlE	TAAATTCATCTAAACGTCACAACTAAA	TGACTGATTGGTTAAATGTGTAACTAA
inlF	AGAAGCGGAAATTTGCATATTAA	ACCAACCATCAAATGTATAACCTTG
inlG	AAACCTCAGTACTACATGTTTTACTTGTAG	TCCTACAAATGTGTAGCCATCTTTC
inlJ	GACATCCCAGATTATACATTAACGACTACT	TTTGCCATTAGCATCCACGTAATTCA
inlC2	GTTTTAATGGTAACTGCTATTCTCGG	AAAACCGCAGTGTAAGCTTCTG
vip	TTACTGTTTCCGTTTTAACTACTTCTATTATTGCCTCACC	CTGGATTTTCTGGAGTGGTAATTTCT

TABLE 3. Summary of MST results for *L. monocytogenes* strains

Clonal complex	Total no.	Human and animal strains							Food and environmental strains	Unknown origin strains
		Brain strains						Non-brain strains		
		Human	Cattle	Sheep	Goat	Other ruminants	Total no.			
A	67	2	22	30	9	1	64	3		
B	14	3	2	6			11	2	1	
C	58	1	1	9	6		17	5	35	1
D	3	1					1		2	
E	2									2
F	4	1		1			2	1	1	
G	3							2	1	
H	3			1	2		3			
SLVs[1]	43	2	2	10	7		21	2	10	10
Total no.	197	10	27	57	24	1	119	15	50	13

[1]SLVs, single locus variants.

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Finally, I would like to dedicate my thesis to my father Romas Balandis.

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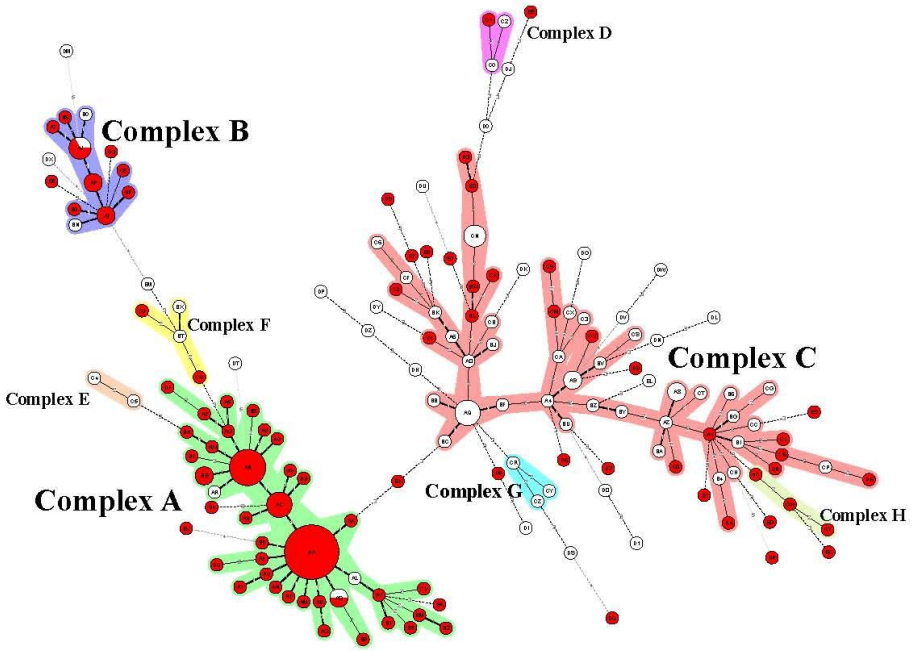
FIGURES

Figure 1

a)



b)

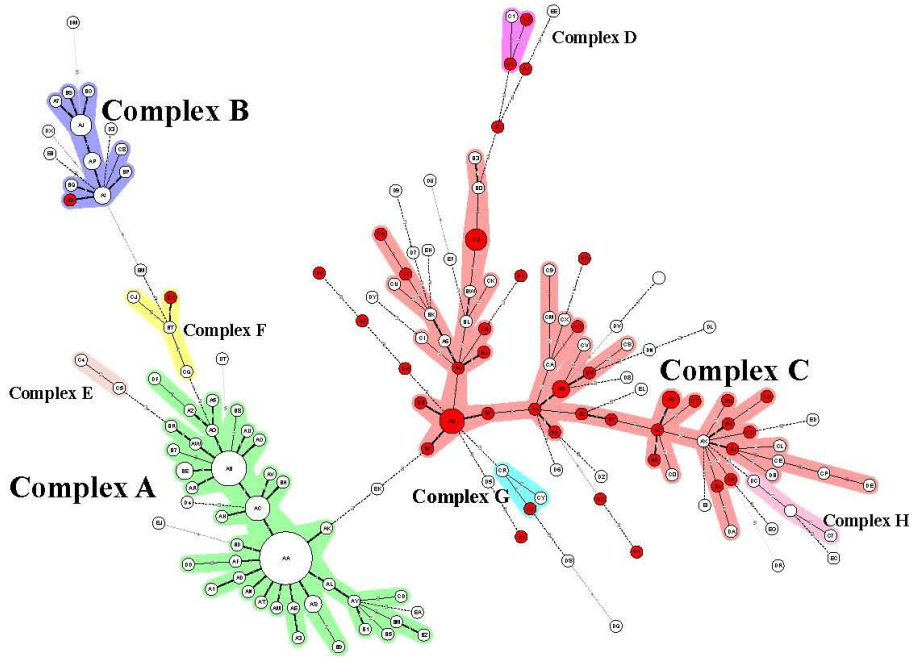


Figure 2

a.


CAA42407 (EGDe) KSEEVNASDFPPPPTDEELRLALPETPMLLGFNAPATSEPSSFEFPPPPT
L86 ActA4 KSEEVNASDFPPPPTDEELRLALPETPMLLGFNAPATSEPSSFEFPPPPT
L11 ActA3 KNEEVNASDFPPPPTDEELRLALPETPMLLGFNAPATSEPSSFEFPPPPT
CAA42407(EGDe) DEELRLALPETPMLLGFNAPATSEPSSFEFPPPPTEDELEIIRETASSLD
L86 ActA4 DEELRLALPETPMLLGFNAPATSEPSSFEFPPPPTEDELEIIRETASSLD
L11 ActA3 ----------EDELEIMRETABSLD
CAA42407 (EGDe) SSFTRGDLASLRNAINRHSQNFSDFPPIPTEEELNGRGGRPTSEEFSSLN
L86 ActA4 SSFTRGDLASLRNAINRHSQNFSDFPPIPTEEELNGRGGRPTSEEFSSLN
L11 ActA3 SSFTSGDLASLRSAINRHSQNFSDFPLMPTEEELNGRGGTPTSEEFSSLN

b.

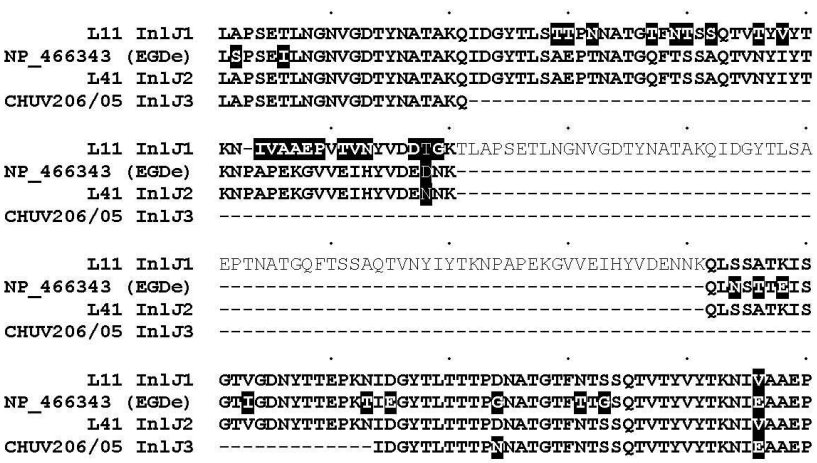
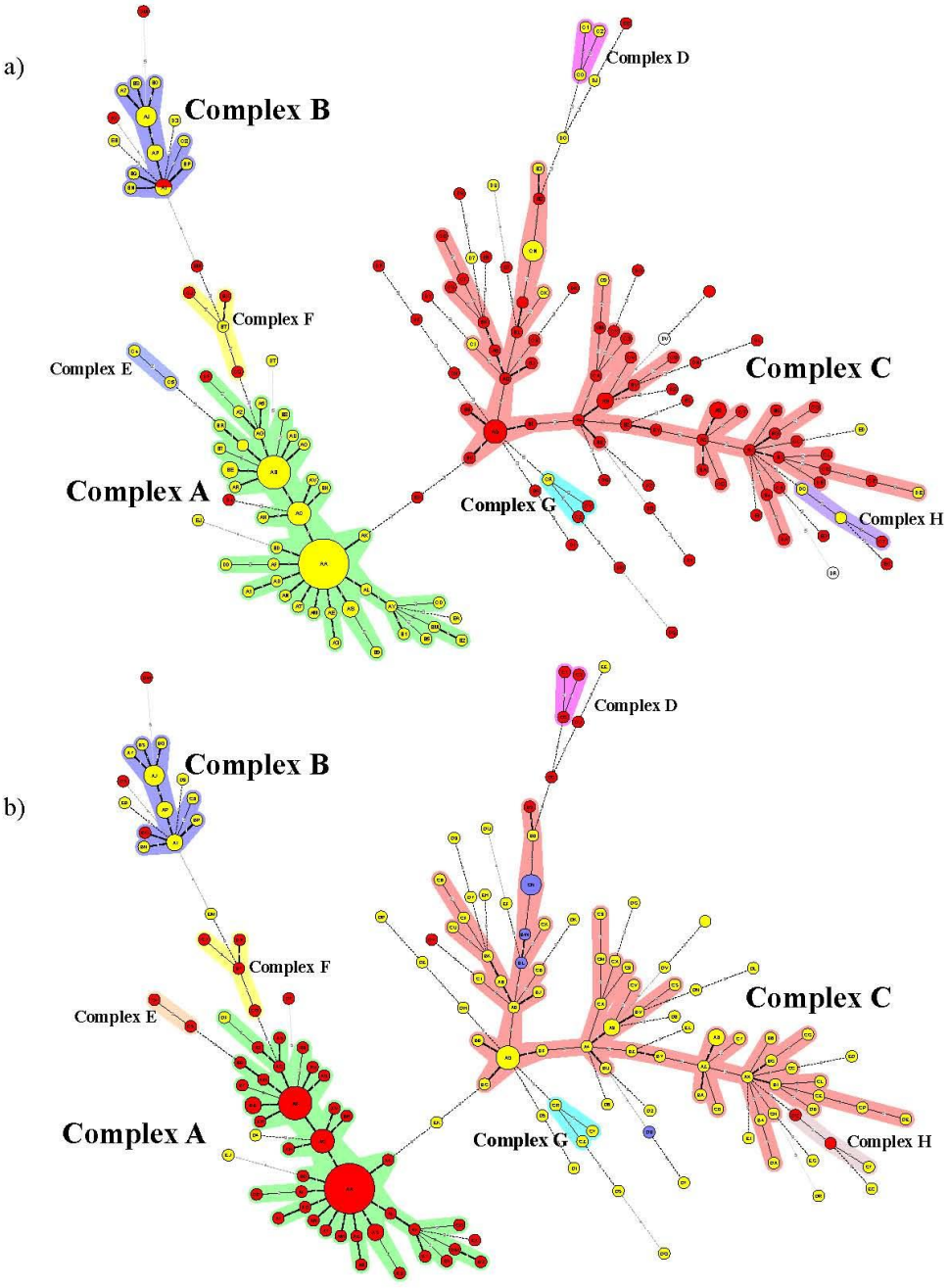

L11 InlJ1 LAPSETLNGNVGDTYNATAKQIDGYTLSTTPNNATGTFNTSSQTVTYVYT
NP_466343 (EGDe) LSPSPILNGNVGDTYNATAKQIDGYTLSAEPTNATGQFTSSAQTVNYIYT
L41 InlJ2 LAPSETLNGNVGDTYNATAKQIDGYTLSAEPTNATGQFTSSAQTVNYIYT
CHUV206/05 InlJ3 LAPSETLNGNVGDTYNATAKQ----------
L11 InlJ1 KN-IVAAEPVTVNYVDDTGKTLAPSETLNGNVGDTYNATAKQIDGYTLSA
NP_466343 (EGDe) KNPAPEKGVVEIHYVDEDNK----------
L41 InlJ2 KNPAPEKGVVEIHYVDENNK----------
CHUV206/05 InlJ3 ----------
L11 InlJ1 EPTNATGQFTSSAQTVNYIYTKNPAPEKGVVEIHYVDENNKQLSSATKIS
NP_466343 (EGDe) ----------QLNSTTEIS
L41 InlJ2 ----------QLSSATKIS
CHUV206/05 InlJ3 ----------
L11 InlJ1 GTVGDNYTTEPKNIDGYTLTTTPDNATGTFNTSSQTVTYVYTKNIVAAEP
NP_466343 (EGDe) GTTGDNYTTEPKTIEGYTLTTTPGNATGTFTTGSQTVTYVYTKNIEAAEP
L41 InlJ2 GTVGDNYTTEPKNIDGYTLTTTPDNATGTFNTSSQTVTYVYTKNIVAAEP
CHUV206/05 InlJ3 ----------IDGYTLTTTPNNATGTFNTSSQTVTYVYTKNIEAAEP

Figure 3


a)
Complex B
Complex D
Complex F
Complex E
Complex C
Complex A
Complex G
Complex H
b)
Complex B
Complex D
Complex F
Complex E
Complex C
Complex A
Complex G
Complex H