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Listeria seeligeri Isolates from Food Processing Environments Form Two Phylogenetic Lineages▽

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Seven different *actA* subtypes forming two phylogenetic lineages could be distinguished by sequencing the *actA* gene of *Listeria seeligeri* isolates from different habitats. Isolates of the two lineages differ in hemolytic as well as phospholipase activities and in the arrangement of the virulence gene cluster. The presence of a serine protease gene resembling *orf2110* of *L. monocytogenes* in some isolates further supports the hypothesis that *L. seeligeri* is subject to ongoing adaptation to changing environments.

The genus *Listeria* comprises the species *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi*, and *L. marthii* (4, 7, 17). Of these only, *L. monocytogenes* (15) and *L. ivanovii* (1, 18) are considered as pathogens. The pathogenicity is closely associated with a virulence gene cluster, although other genes like those coding for internalines are implicated in pathogenesis too (16). Like *L. monocytogenes* and *L. ivanovii*, *L. seeligeri* also carries a virulence gene cluster. Although genes of the virulence cluster are presumably not expressed in a correct or functional fashion (3, 20), *L. seeligeri* has been associated with human infection in one case so far (14). Recent studies reveal exceptions from the traditional classification into pathogenic and nonpathogenic *Listeria* species. *L. innocua* strains carrying a virulence gene cluster similar to that of *L. monocytogenes* have been isolated (6, 22). Moreover, nonhemolytic *L. seeligeri* isolates lacking the major part of the virulence gene cluster have been described (21).

***L. seeligeri* variants.** In this study, a total of 2,492 samples from different habitats of the food chain were investigated with respect to the occurrence of members of the genus *Listeria*. Of 715 isolates obtained from animals (organs and feces), butcher shops (drains and intermediate products), or humans (feces), a total of 44 could be affiliated with *L. seeligeri* (Table 1). The *L. seeligeri* isolates were characterized by sequencing the *actA* gene, which had been used previously for subtyping of *L. monocytogenes* (2, 10, 23, 24). Sequencing of 253 bp of the *L. seeligeri actA* gene with the primers actaf and actar of Volokhov et al. (21) disclosed seven different ActA types (Fig. 1) grouped in two phylogenetic lineages (Fig. 2). While two independent isolates originating from locations not interconnected and 50 km apart are grouped in lineage I, the other 42 *L. seeligeri* isolates from this study are grouped in lineage II. The two lineages can be distinguished also in the case of other genes of the virulence gene cluster (e.g., the seeligeriolysin gene *hly*) and genes not affiliated with the virulence gene cluster (e.g., the 16S rRNA coding genes, the *iap* gene, and the putative protease gene *orf2110*). Isolates of lineage I exhibit higher hemolytic and phospholipase activities on selective agars than those of lineage II (Fig. 3). ActA sequences of lineage II exhibit a high variability on the protein level (from 79.0% to 99.5% identity), resulting in six different *actA* subtypes (Fig. 1). Notable differences are an insert of seven additional amino acids in subtypes 1 and 2, a leucine-proline repeat in subtypes 5 to 7, and a stop codon in the *actA* gene in subtype 4.

Virulence clusters of *L. seeligeri* lineages I and II. Isolates *L. seeligeri* 90 and *L. seeligeri* 187, representing lineage I and lineage II, respectively, exhibit an identical arrangement of the virulence gene cluster with respect to gene organization. Both lineages also show high homology in the housekeeping genes *prs* and *ldh* (100% at the protein level), while other virulence gene products vary with respect to their identity from 81.3% to 99.1% (Table 2). The most significant differences between the lineages are observed with the *dplcB* and *orfY* genes. While the *dplcB* gene of lineage II represents a truncated form of the *plcB* gene, as described previously (3), the *dplcB* gene of lineage I includes 534 additional nucleotides, resembling the full-length adjacent *plcB* gene. The hypothetical translation product, a preproenzyme, shows an identity of only 48.5% to the PlcB phospholipase of the same strain. It differs, however, in five out of nine zinc ion coordinating amino acids from PlcB.

TABLE 1. Occurrence of *Listeria* spp. in organs of animals, feces of humans and animals, and butcher shops[a] in Upper Franconia, Germany

Origin (*n*)	% of samples positive for:				
	Listeria spp.	*L. monocytogenes*	*L. innocua*	*L. welshimeri*	*L. seeligeri*
Animal organs (63)	11.1	6.3	1.6	0.0	3.2
Animal feces (31)	9.7	9.7	0.0	0.0	0.0
Drain (522)	41.8	27.2	14.0	2.5	3.4
Sausage meat (789)	59.1	30.4	23.8	10.4	3.0
Human feces (1,087)	1.9	1.1	0.7	0.4	0.0

[a] Drains and sausage meat.

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Type 1 ACX81169 Ls 90      TNNARNYSQP NNLNHAPVVS TNQGSSKGTL QKQTTVTQVR VPLP~~~~~~ NSKMSSQKQT NQPSNTIQNN KSAAQVNETT SQVS
Type 2 ACX45401 Ls 94700/1B MNNARNFNQL NILNHAPAVS ANQGSSKGTL QKKTIATQVR VPLP~~~~~~ NSKMSSQKQI NKLANTTQNN ~SAAQVNKTT SQVS
Type 3 ACX45410 Ls 53      MNNARNFSQL SNLNHAPAVS ANQGSSKGTL QKQIISTQVR VPLP~~~~~~ NSKMSSQKQI NKSANTTQNN NSVA~~~~~~ ~QVS
Type 4 GQ868302 Ls 226     MNNARNFSQL SNLNHAPAVS ANQGSSKGTL QKQIISTQVR VPLP~~~~~~ NSKMSSQK*I NKSANTTQNN NSVA~~~~~~ ~QVS
Type 5 ACX81188 Ls 187     MNNARNFSQL SNLNHAPAVS ANQGSSKGTL QKQIIATQVR VPLPLP~~~~ NSKMSSQKQI NKSANTTQNN NSVA~~~~~~ ~QVS
Type 6 ACX45431 Ls 13/T3A  MNNARNFSQL SNLNHAPAVS AIQGSSKGTL QKQIIATQVR VPLPLPLP~~ NSKMSSQKQI NKSANTTQNN NSVA~~~~~~ ~QVS
Type 7 ACX45436 Ls 30/T1dB MNNARNFSQL SNLNHAPAVS AIQGSSKGTL QKQIIATQVR VPLPLPLPLP NSKMSSQKQI NKSANTTQNN NSVA~~~~~~ ~QVS
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FIG. 1. Hypothetical protein sequences of the seven identified ActA subtypes of *L. seeligeri* (Ls). The protein sequences correspond to the hypothetical translation products of the 253-bp *actA* gene fragments (Fig. 2). For every subtype, an example is given. Alignments were done with the program BioEdit. The asterisk within the type 4 sequence denominates a stop codon. Gaps are indicated by the "~" symbol.

FIG. 2. Dendrogram showing phylogenetic relationship among *L. seeligeri* (Ls) isolates based on partial *actA* sequences (253 bp) from *L. seeligeri* isolates obtained in Upper Franconia, Germany, and reference strains *L. seeligeri* ATCC 35967 (AY878348) and *L. seeligeri* NRRL (AY510074). The tree was constructed by the minimum evolution method in the MEGA version 4 package. The bootstrap values presented at corresponding branches were evaluated from 1,000 replications. The phylogenetic lineages of the strains are symbolized with Roman numerals and the corresponding *actA* subtypes with Arabic numerals. The bar indicates 1.0% sequence divergence. Strains marked with an asterisk carried a gene similar to the *orf2110* gene of *L. monocytogenes* F2365 (AE017262).

This would result in a nonfunctional enzyme, as an exchange of Trp1 to Leu1 would impair the binding of a zinc ion at site 1.

The serine protease gene *orf2110*. The *orf2110* gene encodes a hypothetical serine protease carrying a V8-like Glu-specific endopeptidase domain (COG3591) (13) as well as three Chw (*Clostridium* hydrophobic tryptophan) domains (12). The *orf2110* gene has been considered specific for *L. monocytogenes* serotypes 4b, 4d, and 4e (7). In this study, a gene showing high similarity to the *orf2110* gene of *L. monocytogenes* (95.6% to *lmof2365_1900* in AAT04669) (11) and to a lesser extent to the analogous gene in *L. welshimeri* (80.2% similarity; *lwe1890* in CAK21308) (5) is described for the first time in *L. seeligeri*. *orf2110* is always located between the genes encoding a hypothetical alkaline protease (*lmof2365_1899* in *L. monocytogenes* F2365 and *lwe1889* in *L. welshimeri* SLCC5334) and a phosphomutase family protein (*lmof2365_1901* in *L. monocytogenes* F2365 and *lwe1891* in *L. welshimeri* SLCC5334). The flanking genes are also present in strains without an *orf2110* gene, like in *L. innocua* (e.g., *lin1984* and *lin1985* in AL592022 *L. innocua* strain Clip 11262) (8) and some *L. seeligeri* strains (Fig. 2). The *orf2110* gene is present in the isolates of lineage I as well as in some isolates of lineage II, primarily in ActA types 4 and 5 (Fig. 2). It is noteworthy that the *orf2110* genes of *L. seeligeri* resemble more closely the genes from *L. monocytogenes*,

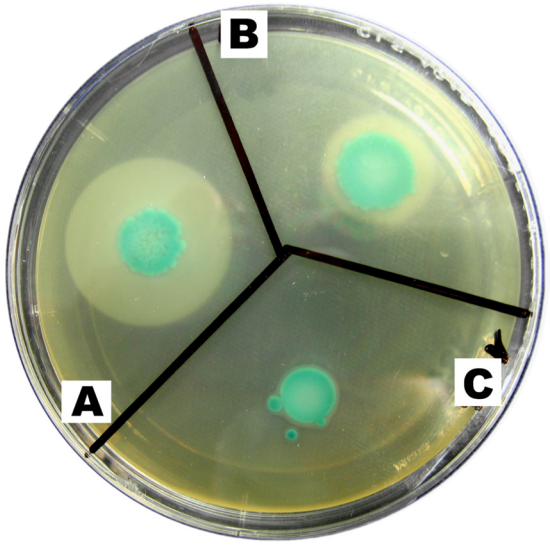

FIG. 3. Phosphatidylinositol-specific phospholipase activity on ALOA agar plates (Merck, Darmstadt, Germany). For analysis of the phospholipase activity, 10 μl of a overnight culture in brain heart infusion (BHI) liquid medium of *L. monocytogenes* 1 (A), *L. seeligeri* 90 (B), and *L. seeligeri* 187 (C) was dropped onto ALOA agar plates and incubated at 37°C for 48 h.

TABLE 2. Similarities and identities between gene and protein sequences of *L. seeligeri* 90 and 187 and *L. monocytogenes* F2365

Lineages compared and comparison parameter	% similarity for comparison[a]																	
	prs[b]	*orfC*	*orfD*	*prfA*	*orfE*	*plcA*	*hly*	*orfK*	*mpl*	*actA*	*dplcB*	*plcB*	*orfY*	*orfX*	*orfI*	*orfP*	*orfB*	*orfA*	*ldh*[b]	*iap*[b,c]
L. seeligeri 90 and 187																				
Similarity (nt)	95.9	95.1	93.7	97.8	96.1	96.3	97.5	93.3	94.2	87.8	34.9	95.1	82.1	98.2	96.8	95.2	100.0	92.7	97.0	95.6
Identity (aa)	100.0	95.0	92.7	99.1	95.5	96.2	98.3	89.2	92.5	81.3	ND	93.5	80.3	98.2	95.3	96.6	100.0	93.2	100.0	95.3
L. seeligeri 187 and *L. monocytogenes* F2365																				
Similarity (nt)	85.4			75.9		65.9	75.7		68.5	47.0		68.3							85.7	39.6
Identity (aa)	95.0			73.8		63.4	81.1		64.3	21.4		62.3							97.0	39.8
L. seeligeri 90 and *L. monocytogenes* F2365																				
Similarity (nt)	87.9			76.4		65.3	75.7		69.7	46.4		68.3							85.7	39.4
Identity (aa)	95.0			73.8		63.7	81.5		65.2	20.8		61.6							97.0	40.2

[a] Nucleotide (nt) similarities and amino acid (aa) identities between gene and protein sequences of the two lineages of *L. seeligeri* isolates 90 (lineage I; GQ862951) and 187 (lineage II; GQ862952) and *L. monocytogenes* F2365 (AE017262). Alignments were done with the program BioEdit. ND, not determined.
[b] Partial sequence.
[c] The *iap* gene encodes the invasion-associated protein P60.

whereas the 16S and 23S rRNA coding genes as well as the *iap* gene are phylogenetically related to *L. welshimeri* (16).

Conclusions. It has been suggested that *L. seeligeri* carries an ancestral form of the virulence gene cluster, which has been deleted from the chromosome of *L. innocua* and *L. welshimeri* (8) and some *L. seeligeri* isolates (21). This cluster would have been subsequently optimized by "pathoadaptive mutations" for the infection and survival in mammals during the evolution of *L. monocytogenes* and *L. ivanovii* (19). It has been hypothesized that the virulence gene cluster of *L. seeligeri* and its low expression are adequate for survival in bacteriovorous protozoae like *Tetrahymena* (9). However, our results suggest that *L. seeligeri* is subject to ongoing adaptation to changing environments. Accordingly, lineage II appears to be better adapted to natural environments, as indicated for example by the deletion in the *dplcB* gene, the stop codon within the *actA* gene, or the loss of the entire virulence gene cluster (21). On the other hand, lineage I might be better adapted to the mammalian hosts, as indicated by higher hydrolytic activities. The concomitant deletion in the *orfY* gene might also contribute to survival in mammalian hosts as this gene is lacking in *L. monocytogenes* too.

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