A *Listeria monocytogenes* strain is still virulent despite non-functional major virulence genes: Optical Mapping shows a potential mechanism

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In previous studies 24 naturally occurring low-virulence *Listeria monocytogenes* strains were identified using a method that combines a plaque-forming assay with subcutaneous injection into the left hind footpad of mice[1]. Based on their phenotypic characteristics, these low-virulence strains were assigned by cluster analysis to one of four groups[2]. The 11 strains belonging to Group I exhibit a mutated PrfA[3] whereas 5 out of the six strains belonging to Group III have causal mutations in *plcA*, *inlA* and *inlB* genes[4].

New strains exhibiting the same PFGE specific profile than the low-virulence Group III strains have been identified and characterized. All were low-virulence strains, beside the A23 strain which has been characterized as a virulent strain in the mouse model[5]. Further characterizations of this strain were performed.

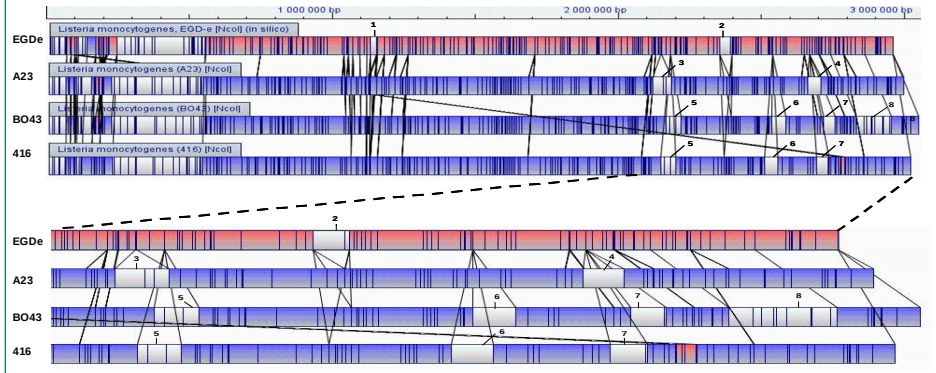
Phenotypic and genotypic characterization

Strains	Plaque-forming assay Mean (log)* ± SD	Invasion assay (at 3h) Mean (%) ± SD	Subcutaneous infection (spleens)		Enzyme activities		Mutations			
			Mean (log) ± SD	Infected/Total	PI-PLC	PC-PLC	InlA	InlB	PI-PLC	Mpl
EGDe	5.60 ± 0.22	2.00 ± 0.97	5.54 ± 0.46	5/5	8.13 ± 2.71	6.37 ± 0.41	wild-type	wild-type	wild-type	wild-type
416	0	0.06 ± 0.05	0	0/5	0.39 ± 0.01	4.36 ± 0.31	Truncated (nt77)	Ala117Thr Val132Ile	Thr262Ala	wild-type
BO43	0	0.45 ± 0.17	2.53	1/5	0.70 ± 0.54	5.35 ± 0.71	Truncated (nt77)	Ala117Thr Val132Ile	Thr262Ala	wild-type
A23	4.92 ± 0.38	0.11 ± 0.10	2.88 ± 1.02	10/10	1.74 ± 0.64	0	Truncated (nt77)	Ala117Thr Val132Ile	Thr262Ala	Leu220Phe

* log(number of plaques per 10^7 c.f.u.. deposited)

The A23 strain exhibited the same mutations than the Group III strains (416 and BO43) in the *inlA*, *inlB* and *plcA* gene, and a supplementary mutation in the *mpl* gene, involved in the maturation of the PC-PLC.

Optical mapping



In the pair-wise alignments, lines connecting two chromosomal maps indicate a discontinuity in the alignment of fragments. Chromosomal inversions are indicated by crossed alignment lines between paired maps and are highlighted in purple. Unaligned restriction fragments, representing differences between two aligned chromosomes, are shown in white; blue indicates aligned restriction fragments.

Aligned optical maps were performed for Group III strains (BO43 and 416) and A23 strains compared to the *in silico* reference EGDe map. The EGDe map is approximately 20% different from the maps of the Group III and A23 strains. This difference could be explained by the fact that the EGDe strain (1/2a) represents an intermediate evolutionary state between 1/2a and 1/2c serotypes[6].

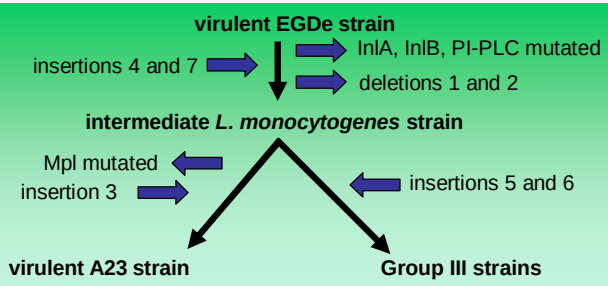
The fragments 3 and 4 represent inserted fragments in the A23 chromosome. The fragments 5, 6 and 7 represent inserted fragments in the chromosomes of the BO43 and 416 strains. A supplementary fragment 8 is inserted in the chromosome of the BO43 strain.

The Group III strains are highly homogeneous. The A23 strain is closely related to Group III strains. Indeed the insertion of the fragment 4 is located at the same place as the fragment 7. The fragment 3 present in the A23 strain is different from the fragment 5, present in the Group III strains and could explain the increase of virulence of the A23 strain. Contrary the fragment 6 present in the Group III strains could explain the decrease of virulence of these strains compared to the A23 strain. Indeed the annotation of the EGDe strain indicates that this insertion could be in the *clpP* gene involved in the rapid adaptive response of intracellular pathogens during the infectious process.

Conclusion

Despite mutations in the major virulence genes (*inlA*, *inlB*, *mpl* and *plcA* genes), the A23 strain was virulent in a plaque–forming assay and in a mouse model. It was closed to the Group III strains by MLST and PFGE analyses, exhibiting the same mutations in the *inlA*, *inlB* and *plcA* genes. The analysis by optical mapping gives indications that could be further investigated. The hypothetic evolution of the A23 strain suggested that it diverged from an intermediate strain which evolved by acquisition of point mutations and genes.

Phylogenetic analysis of these strains could be fruitful to understand the evolution of virulence trait as well as plasticity of virulence genes.



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(4) Témoin S *et al*. (2008) Microbiology 154:939-948. **(5)** Roche S M *et al*. (2009). J Inf Dis. 200:1944-1948. **(6)** Ragon M *et al* (2008) PLoS Pathog. 4:e1000146

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