Assessing the virulence potential of different *Listeria monocytogenes* Clonal Complexes with *Galleria mellonella* larvae model

Mariana Sousa [1*], Sónia Silva[2,3], Daniela Araújo[2], Joana Castro[2,3], Gonçalo Almeida[2], Catarina Ventura[1], Rui Magalhães[1], Vânia Ferreira[1], Paula Teixeira[1]

[1] Universidade Católica Portuguesa, CBQF - Centre of Biotechnology and Fine Chemistry– Associate Laboratory, Faculty of Biotechnology, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

[2] National Institute for Agrian and Veterinary Research (INIAV, I.P.), Vairão, 4485-655 Vila do Conde, Portugal

[3] Centre of Biological Engineering (CEB), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

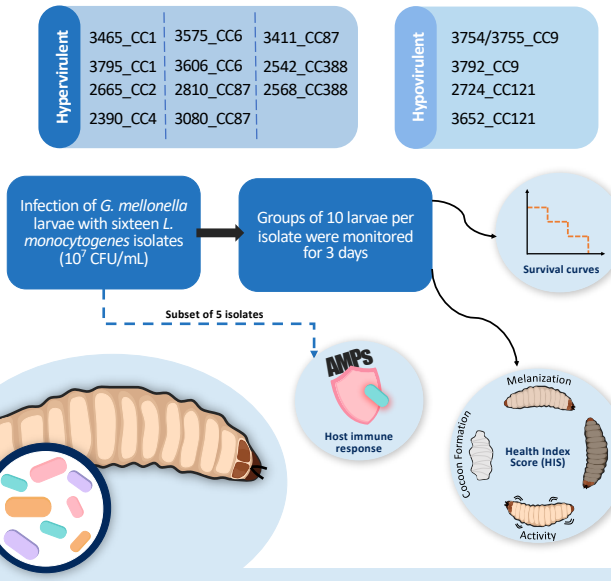
*marsousa@ucp.pt

Introduction and Objective

Listeria monocytogenes is a pathogen causes infection mainly through food contamination. This species is well-known for its diversity, exhibiting differential virulence potential within strains from different clonal complexes (CCs). These can be defined as hypervirulent CCs, that are highly frequent among clinical cases and cause severe clinical outcomes (i.e. maternal-neonatal and central nervous system infections), or as hypovirulent CCs, that are associated with food and food processing environments and present attenuated virulence, causing disease in highly immunocompromised individuals. Many methodologies have been employed to study this differentiated pathogenicity, with *Galleria mellonella* larvae emerging as an *in vivo* infection model [1, 2]. The purpose of this study was to assess the pathogenic potential of *L. monocytogenes* isolates from eight distinct CCs, either hyper- and hypovirulent, through an *in vivo* infection and further analysis of its impact on the immune response of *Galleria* larvae .

Methodology

Sixteen *L. monocytogenes* isolates from **hypervirulent** (CC1, CC2, CC4, CC6, CC87 and CC388) or **hypovirulent** (CC9 and CC121) CCs were selected for the purposes of this study. An infectious dose of 1x10^7 CFU/mL of *L. monocytogenes* was injected in the hindmost left proleg, into the hemocoel of the larvae.



Results

The survival curves and the health index scores of the *G. mellonella* larvae infected with the 16 selected *L. monocytogenes* isolates are represented, respectively, in Figure 1A and 1B. The gathered results showed that:

- The two *L. monocytogenes* isolates that caused the **highest and lowest survival rates** are from **hypervirulent CCs**;
- Significant **differences intra-clonal complex** were observed within the CC6;
- Isolates from CC9 exhibited a **hypervirulent phenotype** when injected into *G. mellonella* larvae;
- At all time points, a decrease in the HISs was observed for larvae injected with each isolate. The observed HIS pattern was similar to that noted on the survival curves.

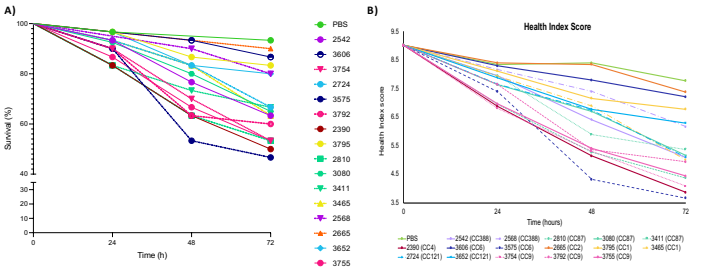


Figure 1. A) Survival curves of *Galleria mellonella* larvae infected with 10^7 CFU/mL, along 72 h of infection. **B)** Evaluation of the health index of *G. mellonella* inoculated with *L. monocytogenes* strains at 10^7 CFU/mL during three time points (24h, 48h and 72h post-infection).

Considering the pathogenic capacity of *L. monocytogenes* strains, a subset of five isolates was selected to pursue with further analysis of the host's immune response based on the expression of the genes coding for antimicrobial peptides (AMPs). The obtained results showed an **AMPs overexpression** in strains that caused **lower survival rates**.

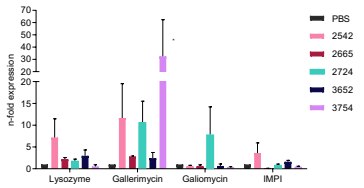


Figure 2. Transcription levels of *G. mellonella* worms' immune-related genes, 48 h post-infection with *L. monocytogenes*. The expression levels of some antimicrobial peptides (lysozyme, gallerimycin, galiomycin and IMPI) were measured after infection with 10^7 CFU/mL after 48 h of infection.

Conclusions

The results showed that **no correlation** between **hyper- or hypovirulent CCs** and **survival rates or HIS**, as isolates causing higher and lower survival were from hypervirulent CCs. Additionally, the virulence potential of *L. monocytogenes* strains seems to be **strain-dependent** upon infection of *G. mellonella* larvae. Further studies need to be conducted with a larger sample size to draw more robust conclusions. Additionally, the expression of AMPs should be monitored throughout the infection process along with bacterial cells levels.

References

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[2] Cardenas-Alvarez, M. X., *et al.* (2019). Evidence of hypervirulence in *L. monocytogenes* clonal complex 14. *Journal of Medical Microbiology*, 68(11), 1677-1685.

Acknowledgements

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