

In the quest for reliable tools to explore the virulence potential of *Listeria monocytogenes* clonal complexes

Mariana Sousa*, Rui Magalhães, Vânia Ferreira, Paula Teixeira

Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado
Escola Superior de Biotecnologia, Rua Diogo Botelho, 1327, 4169-005, Porto, Portugal. pteixeira@porto.ucp.pt
*marsousa@ucp.pt

Introduction and Objective

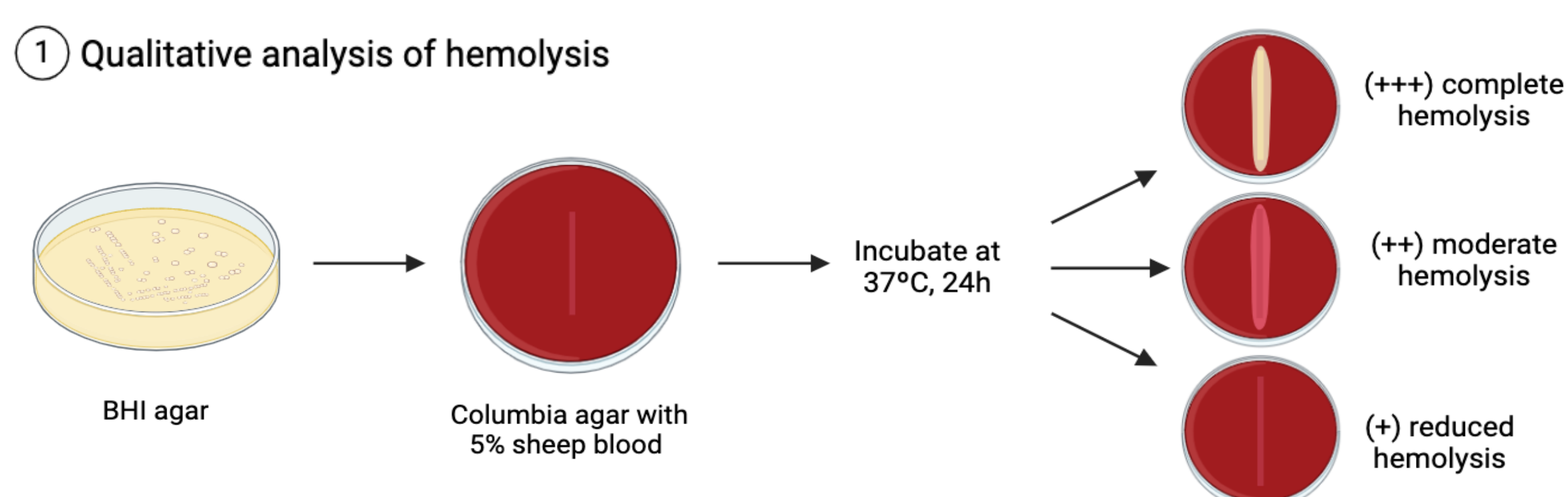
Listeria monocytogenes is a foodborne pathogen that causes human listeriosis. This pathogen is characterized by an intra-species heterogeneity so its strains can be grouped into clonal complexes (CCs) that are defined either as **hypervirulent** – mainly associated with clinical cases or **hypovirulent** – associated with food or food processing environments and with infections in susceptible groups [1]. The impact of *L. monocytogenes* extends beyond health concerns to economic implications since detection of contaminated food results in production interruptions and product recalls. Considering the persistence of *L. monocytogenes* in the food industry, there is an urgent need to define reliable biomarkers and to determine whether CC characterization is robust enough to discriminate high-risk strains. *Listeria monocytogenes* has a typical β -hemolytic profile, which is outlined by crucial virulence factors (*hly* and *prfA* genes), however, some strains exhibiting non-hemolytic or attenuated hemolysis have been reported [2]. **The aim of this work was to investigate if these phenotypes could be associated with hypovirulent CCs.**

Methodology

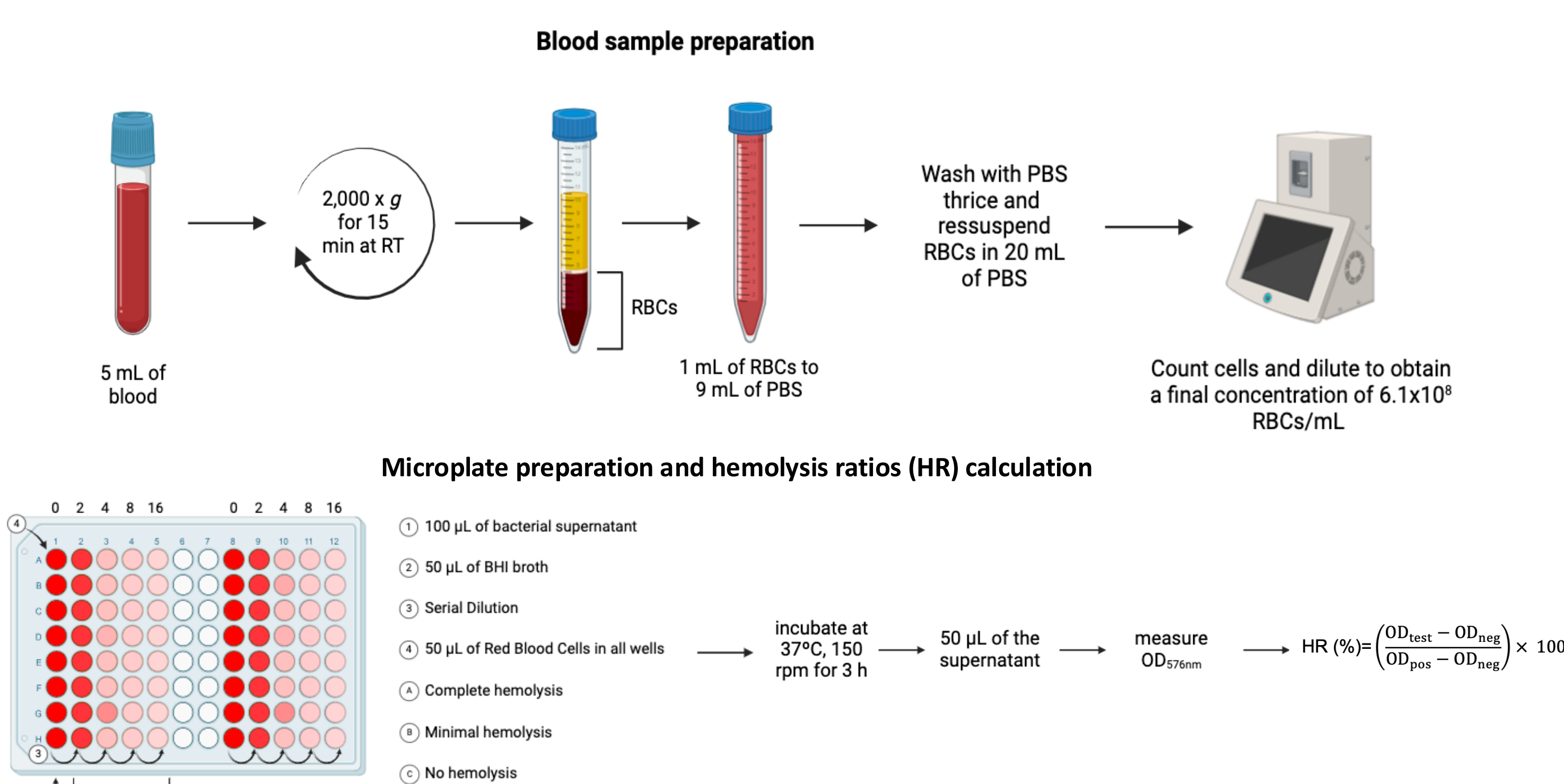
Sixteen *L. monocytogenes* isolates from **hypervirulent** (CC1, CC2, CC4, CC6, CC87 and CC388) or **hypovirulent** (CC9 and CC121) clonal complexes were selected. Additionally, five strains were selected to gene expression studies.

Strain ID	Strain ID	Strain ID	Strain ID
3465_CC1	3575_CC6	3411_CC87	3754/3755_CC9
3795_CC1	3606_CC6	2542_CC388	3792_CC9
2665_CC2	2810_CC87	2568_CC388	2724_CC121
2390_CC4	3080_CC87		3652_CC121

1 Qualitative analysis of hemolysis



2 Qualitative analysis of hemolysis



3 Virulence gene expression



Results

- Blood agar plates

- All *L. monocytogenes* exhibited a **hemolytic phenotype**.
- Variability in the hemolytic activity within *L. monocytogenes* strains.
- The strains 2724, 3465 and 3606 presented a **strong** hemolytic activity.

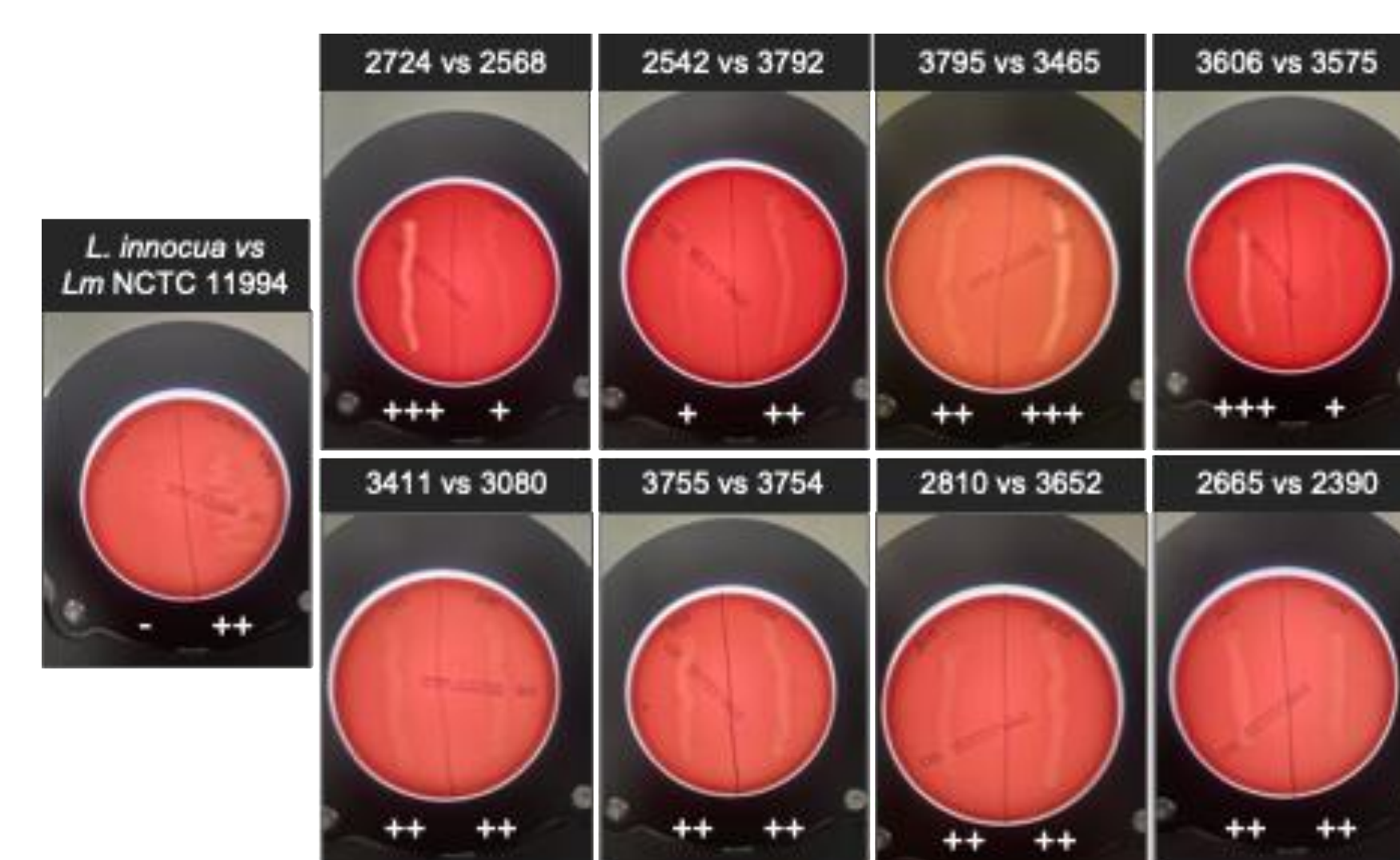


Figure 1. Hemolysis assays in blood agar plates. The isolates ID is presented above each figure. The hemolysis was classified as (-) in the absence of hemolysis.

- Microplate technique

The majority of isolates presented a minimal and complete hemolytic unit of 8 and 4, respectively. The strain **2724 strain** stood out with an **increased lysis of the RBCs** and reduced levels of deposition of these cells (Fig. 2), which is corroborated by an increase HR% (Table 1).

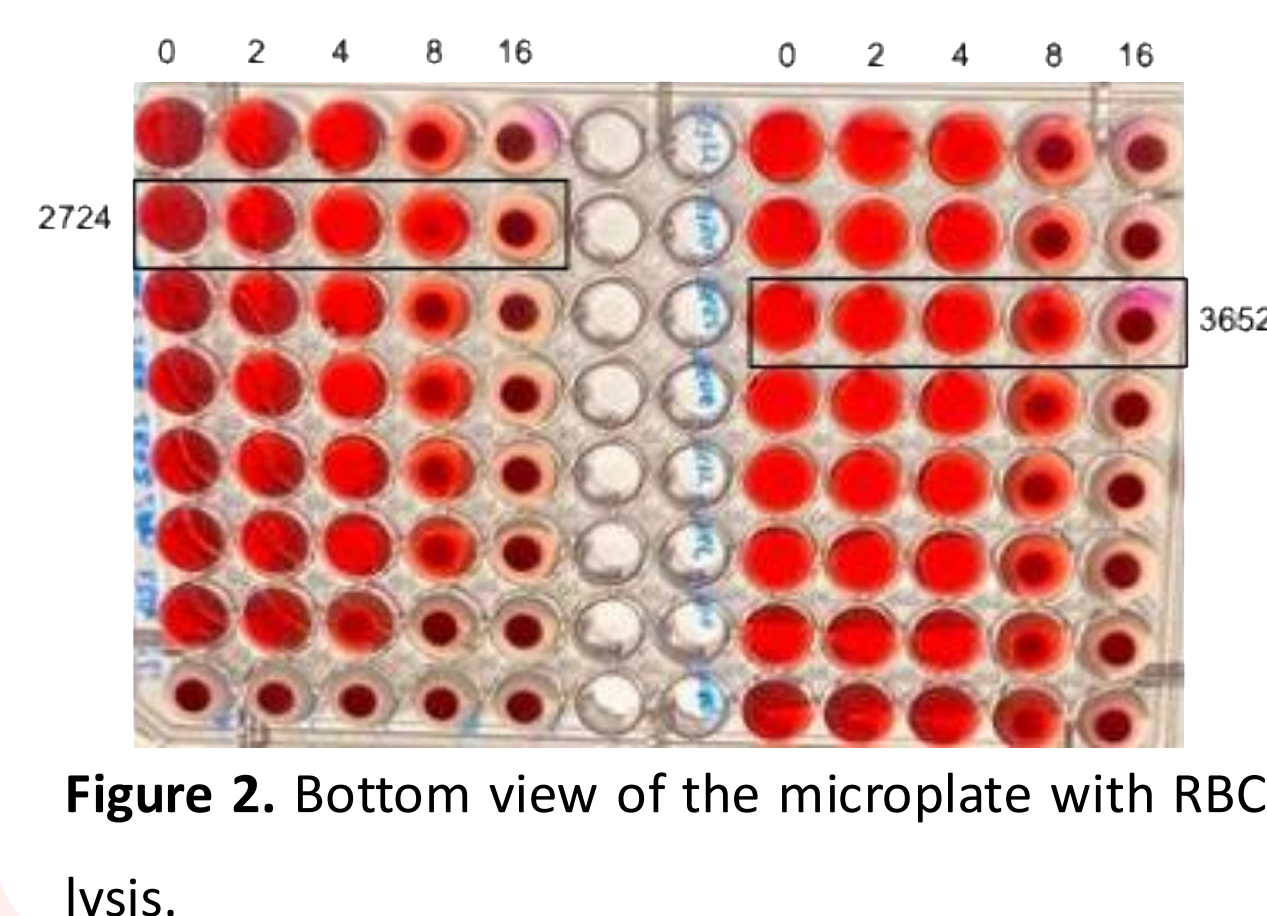


Table 1. *L. monocytogenes* hemolysis ratios (%). The OD_{576nm} values obtained in titer 8 column were used for these calculations.

Strains and Controls	HR (%)	Strains and Controls	HR (%)
SDS 0.1%	100.0	3575	61.9
2724	93.8	2665	57.8
3652	80.4	2810	44.9
2568	74.9	3755	40.7
3606	74.4	3754	40.7
3080	72.1	3792	16.3
3411	67.1	3795	15.4
2542	66.0	EGDe	8.5
3465	64.8	<i>L. innocua</i>	0.5
2390	64.3	BHI	0.0

- Virulence gene expression

There was a clear increase in the transcript levels of strain 2724 *inA*, *actA* and *hly*, with increases of ~19-fold, ~25-fold and ~14-fold, respectively.

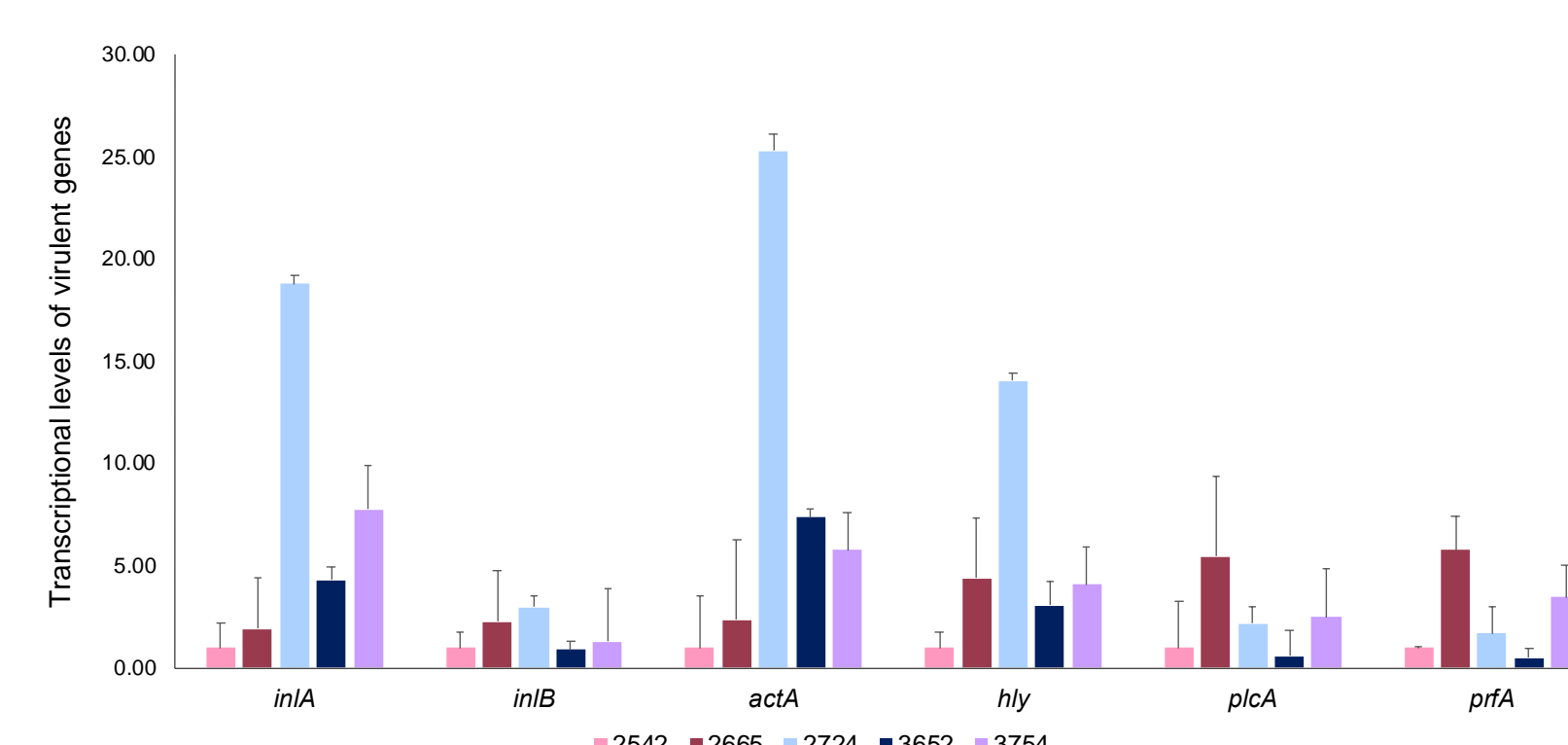


Figure 3. Transcription levels of some genes essential for the infection mechanism of *L. monocytogenes* after growth on BHI broth. Data are presented as mean \pm standard deviation.

Conclusions

No correlation between increased hemolysis and hypervirulence

Overexpression of some virulence genes can lead to reduced infection periods

Hemolysis does not seem to be a reliable tool to study the hyper- and hypovirulence

References

- Maury, M. M., et al. (2016). Uncovering *L. monocytogenes* hypervirulence by harnessing its biodiversity. *Nature Genetics*, 48(3), 308-313.
- Maury, M. M., et al. (2017). Spontaneous loss of virulence in natural populations of *Listeria monocytogenes*. *Infection and Immunity*, 85(11), e00541-00517.

Acknowledgements

This work was supported by National Funds from FCT - Fundação para a Ciência e a Tecnologia through project GenoPhenoTraits4Persistence - Genomic and phenotypic traits contributing to persistence of *Listeria monocytogenes* in food processing environment (PTDC/BAA-AGR/4194/2021)