

# Differentiation between persistent and sporadic *Listeria monocytogenes* through growth kinetics

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## Introduction

*Listeria monocytogenes* is a ubiquitous, facultative anaerobic, Gram-positive foodborne pathogen. This aetiological agent can cause severe illness in vulnerable populations. Furthermore, and contrasting many of its innocuous neighbours, this pathogen is known for persisting in various environments, including food processing environments (FPEs). Interestingly enough, only certain strains are routinely isolated from these FPEs while others are sporadically isolated. One possible explanation for this persistence has been attributed to the formation of cell subpopulations capable of withstanding adverse conditions, which may encompass high salinity, low temperature, or low pH.

## Methodology

To determine differences in fitness, a three-level three condition full factorial design was implemented, with 18 *L. monocytogenes* strains being grown in culture media with combinations of pH (adjusted with lactic acid), NaCl, and temperature.

Bacterial growth was evaluated by tracking OD600 through incubation with a UV/VIS microplate spectrophotometer.



**Table 1.** Central composite design arrangement for *Listeria monocytogenes* growth under extreme stress conditions, variables and levels.

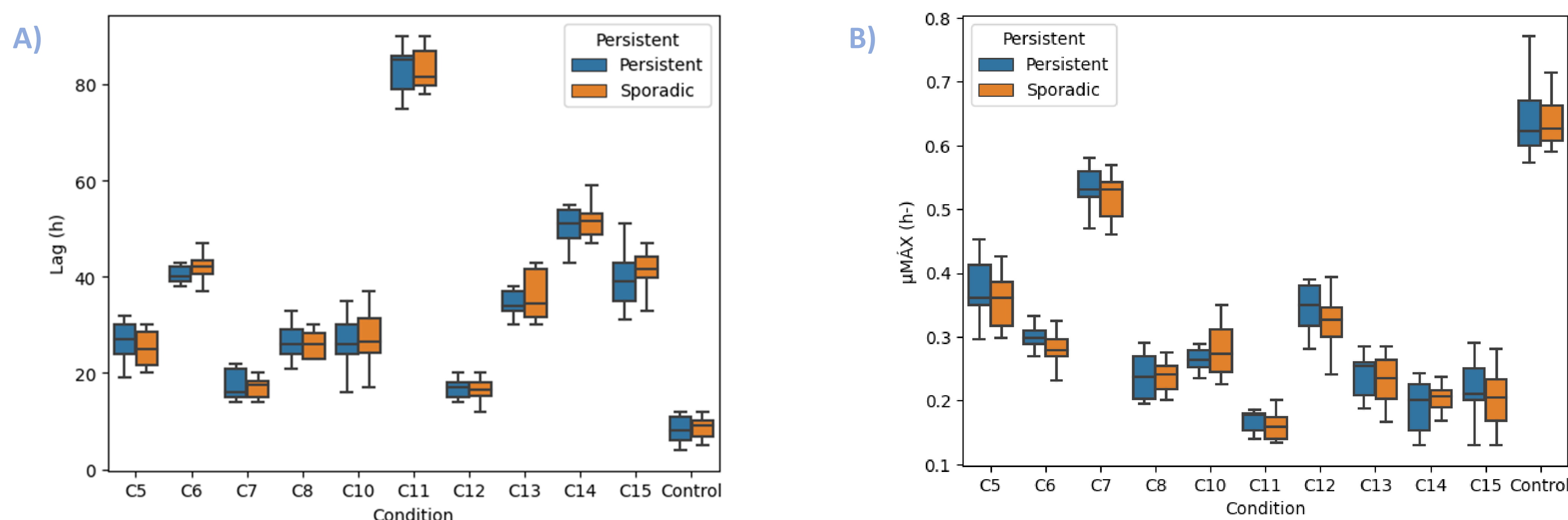
Coded variable levels				
Variable	Symbol	-1	0	1
Salt Concentration (% v/w)	NaCl	2.50	4.00	8.00
Temperature (°C)	Temp	11	22	30
pH	pH	5	6	7

Variable levels				Responses			
Run	pH	Temp	NaCl	pH	NaCl (% v/w)	Temperature (°C)	Incubation Period (h)
C1	-1	-1	-1	5	2.5	11	168
C2	-1	-1	1	5	8.0	11	168
C3	-1	1	-1	5	2.5	30	168
C4	-1	1	1	5	8.0	30	168
C5	1	-1	-1	7	2.5	11	72
C6	1	-1	1	7	8.0	11	168
C7	1	1	-1	7	2.5	30	24
C8	1	1	1	7	8.0	30	72
C9	-1	0	0	5	4.0	22	168
C10	1	0	0	7	4.0	22	72
C11	0	-1	0	6	4.0	11	72
C12	0	1	0	6	4.0	30	48
C13	0	0	-1	6	2.5	22	48
C14	0	0	1	6	8.0	22	120
C15	0	0	0	6	4.0	22	72

## Results

- From the 18 tested strains, we did not observe statistically meaningful differences between the persistent and transient *L. monocytogenes* groups.
- Regarding the three tested, low pH and low temperature were the most impactful variables in the growth kinetics of our isolates.



**Figure 1.A)** Relative lag time. The y axis represents the lag time (defined as the last time point before the growth curve entered exponential growth). In orange and blue, respectively, we have represented the average lag time value for the sporadic isolates and persistent isolates. **B)** Maximal growth rate. The y axis represents the maximum growth rate (calculated from the maximum slope).

## Conclusion

- *L. monocytogenes* persistent and transient groups did not present any meaningful differences in their growth kinetics when subjected to food processing related stresses.
- Strain variability was more profound when compared between persistent and sporadic strains.
- Persistence events may not be correlated with better fitness in growth, further studies should be performed.

## Acknowledgements

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