

Survival of *Listeria monocytogenes* strains from different ecological niches through acidic conditions

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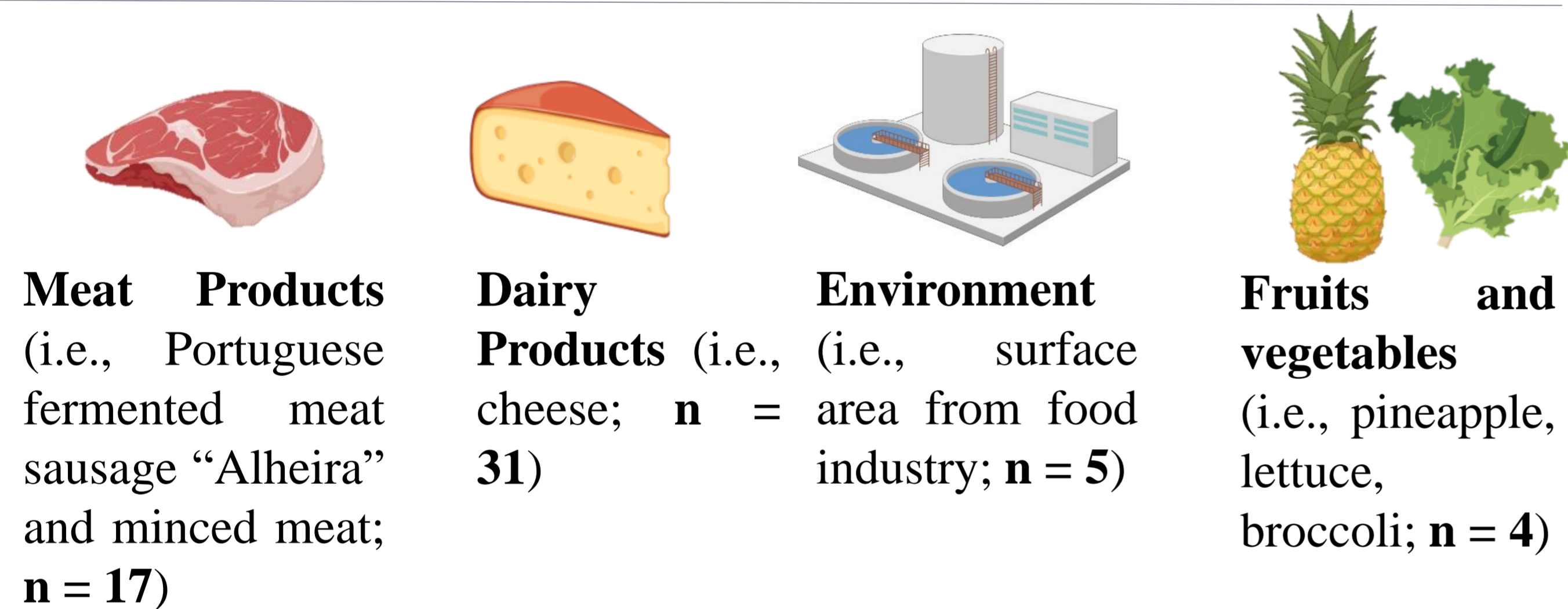
Introduction

Listeria monocytogenes is the etiological agent of human listeriosis, a serious infection with a high fatality rate that can exceed 40% in the European Union^[1]. This Gram-positive bacterium can be found in different ecological niches including soil, stream water, sewage, and plants^[2], and is noteworthy for its persistence in food-manufacturing environments due to its high environmental tolerance. *Listeria monocytogenes*, which occurs as a saprophyte in nature, can easily switch to its infectious life and become a lethal intracellular pathogen when ingested in contaminated food^[3]. As a result, this pathogen has become a significant concern for public health and food safety, with far-reaching economic consequences that emphasize the urgency of addressing its impact^[4,5]. From a risk analysis perspective, it is also important to assess the virulence potential of strains from different niches that represent a continual risk of contamination. This study aimed to evaluate the survival of *Listeria monocytogenes* strains from different ecological niches under acidic conditions, simulating stomach digestion.

Methods

The stomach digestion, normally assisted with enzymes (i.e., pepsin), followed a simple/preliminary methodology based on the INFOGEST static *in vitro* simulation.

A. Model and source of *L. monocytogenes* used



57 sequenced
L. monocytogenes
strains

Washed cells

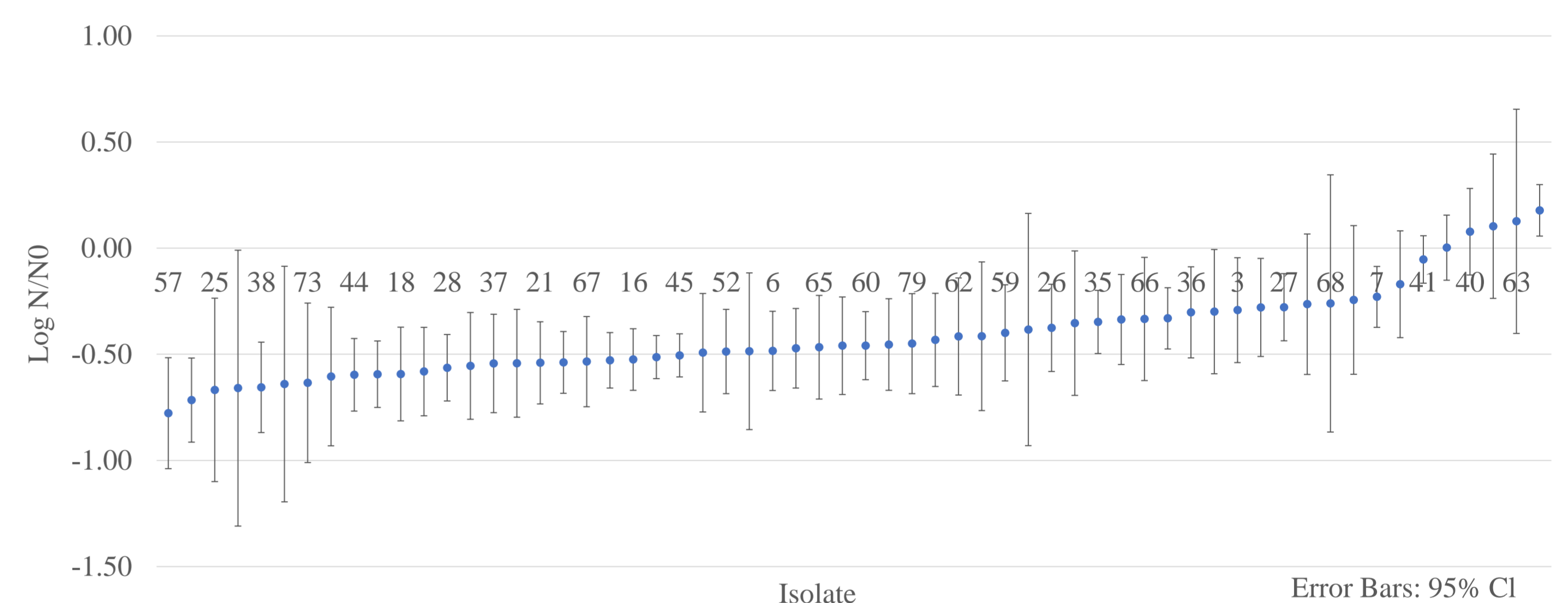
B. Screening to evaluate their capacity to survive in an acidic condition:

- Stomach digestion 1:1 (v/v) with buffered peptone water (BPW) adjusted to pH3 with HCl 6M
-
- t0** Sample taken immediately after addition (N0)
- t2** 2 hours of incubation in a water bath at 37 °C with agitation (90 rpm) (N)

Results

Three independent assays in duplicate were performed and the results were represented as Log N/ N0.

A. Screening of 57 isolates after 2 hours on acidic fluid (pH 3)



B. Lowest and highest values obtained after 2 hours of acidic digestion

Model	Source of <i>L. monocytogenes</i>	Log N/N0	
		Highest	Lowest
Environmental surfaces		0.00 ± 0.15	-0.52 ± 0.14
Dairy Products	Cheese	0.13 ± 0.50	-0.78 ± 0.25
Meat Products	“Alheira”	0.18 ± 0.12	-0.67 ± 0.41
	Minced Meats	0.08 ± 0.19	-0.66 ± 0.20
Fruits and Vegetables	Pineapple		-0.66 ± 0.62
	Lettuce		-0.59 ± 0.21
	Broccoli		-0.24 ± 0.33

- Regardless of the model used and the source of the pathogen, it is not possible to discriminate between resistant or sensitive isolates to acidity (pH 3);
- Strains isolated from matrices with more protein or more fat (meat and dairy products) appear to have greater resistance. But this pattern was not observed for all isolates;
- No apparent differences were observed between the models used or the sources of the pathogen.

Conclusions

The methodology used for screening resistance to acidity, simulating the acidic conditions of the stomach, which was based on a pH 3, as recommended by the INFOGEST protocol, does not seem to be the best option. To recreate a harder hurdle, the evaluation of survival at lower pH (between 1.5 to 2.5) would be preferred in order to obtain greater discriminatory results.

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Acknowledgments

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