

Influence of *Listeria innocua* on the growth of *Listeria monocytogenes*



CATÓLICA
UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO
Escola Superior de Biotecnologia

Carvalho A., Eusébio C., Silva J., Gibbs P., Teixeira P.*

CBQF/Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Porto, Portugal

*corresponding author: pcteixeira@mail.esb.ucp.pt

INTRODUCTION

Listeria monocytogenes represents an important foodborne pathogen which causes listeriosis, a serious invasive illness in humans (Farber and Peterkin, 1991). Its detection is crucial within the food industry because consumption of contaminated raw and/or processed food products such as meat, poultry, seafood, dairy products and vegetables, is the cause of 99% of all listeriosis cases (Schlech, 2000). It usually involves selective enrichment procedures however, several research reports have demonstrated that the presence *L. innocua* may mask *L. monocytogenes*, leading to a false negative result for the presence of *L. monocytogenes* (Cornu et al. 2002).

The aim of the present work was to evaluate the influence of *L. innocua* on the growth of *L. monocytogenes*.

METHODOLOGY

► **Monitoring growth of *L. monocytogenes* strains in the presence of *L. innocua* in three different mixtures:**

A) *L. monocytogenes*_1339 and *L. innocua*_11288

B) *L. monocytogenes*_1340 and *L. innocua*_11288

C) Six strains of *L. monocytogenes* (1339, 1340, 1334, 1792, 999, 1336) and *L. innocua*_2030c

In non-selective medium
(TSBYE)

L. monocytogenes (10^4 cfu/ml) and *L. innocua* (10^4 cfu/ml)

In food matrix (pasteurized milk) artificially contaminated with different conditions undergoing enrichment culturing according to ISO 11290-1 reference method (Anonymous, 2004).

Condition	Inoculum
1	<i>L. monocytogenes</i> (10^2 cfu/ml) and <i>L. innocua</i> (10^2 cfu/ml)
2	<i>L. monocytogenes</i> (10^4 cfu/ml) and <i>L. innocua</i> (10^4 cfu/ml)
3	<i>L. monocytogenes</i> (10^4 cfu/ml) and <i>L. innocua</i> (10^2 cfu/ml)
4	<i>L. monocytogenes</i> (10^2 cfu/ml) and <i>L. innocua</i> (10^4 cfu/ml)

► **Enumeration:** Aliquots of the different mixtures that were obtained at each defined interval were serially diluted and plated using the drop counting technique onto the Agar Listeria Ottaviani Agosti plates and incubated at 37°C for 18 – 48 h.

► **Detection of any inhibitory activity produced by *L. innocua* against *L. monocytogenes* was screened by the spot on lawn method (Tagg et al., 1976).**

CONCLUSION

It was observed inhibition of *L. monocytogenes* by *L. innocua* but the reverse was also observed. More *L. monocytogenes* and *L. innocua* strains should be tested in order to understand the heterogeneity in strains' behavior. Furthermore no inhibitory activity caused by bacteriocins was found and the growth rate decrease when there was inhibition only happened in some cases. An alternative explanation could be that of 'quorum sensing' (QS), that is regulation of gene expression in response to cell population density, in this case resulting in an inhibition of growth of a population of bacteria when a certain number are present, usually ca. 10^6 CFU/mL.

REFERENCES

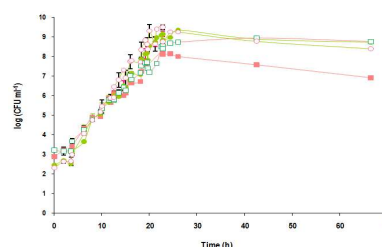
Anonymous (2004) Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Detection and Enumeration of *Listeria monocytogenes*. ISO 11290-1: 1996/Amd.1:2004(E), International Organization for Standardization, Geneva.
Besse NG, Audinet N, Kérouanton A, Collin P, Kalmokoff M (2005) Evolution of *Listeria* populations in food samples undergoing enrichment culturing. Int J Food Microbiol 104: 123-134.
Cornu M, Kalmokoff M, Flandrois JP (2002) Modelling the competitive growth of *Listeria monocytogenes* and *Listeria innocua* in enrichment broths. Int J Food Microbiol, 73:261-274.
Farber JM, Peterkin PI (1991) *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev, 55:476-511
Schlech WF (2000) Foodborne listeriosis. Clinical Infect Dis, 31:770-775.
Tagg JR, Dajani AS, Wannamaker LW. (1976) Bacteriocins of Gram-positive bacteria. Bacteriol Rev ;40:722-56.
Yokoyama E, Maruyama S, Katsube Y, Mikami T (2005) Influence of bacteriocin-like substance, generation times, and genetic profiles of *Listeria innocua* on the isolation of *Listeria monocytogenes*. Comp Immunol Microbiol Infect Dis. pp:1-10.

ACKNOWLEDGEMENTS

This work was supported by FCT PTDC/AGR-ALI/64662/2006 .

RESULTS AND DISCUSSION

► **Growth curves in non-selective medium:**



Growth of *L. monocytogenes* in TSBYE was inhibited by *L. innocua* only in mixture B (Fig. 1).

Fig. 1 Growth curves in TSBYE of *L. monocytogenes*_1340 with *L. innocua*_11288. Error bars indicate variability between assays (● - *L. innocua* in mixture; □ - control of *L. innocua*; ■ - *L. monocytogenes* in mixture; □ - control of *L. monocytogenes*).

► **Growth Curves in food matrix artificially contaminated undergoing enrichment culturing**

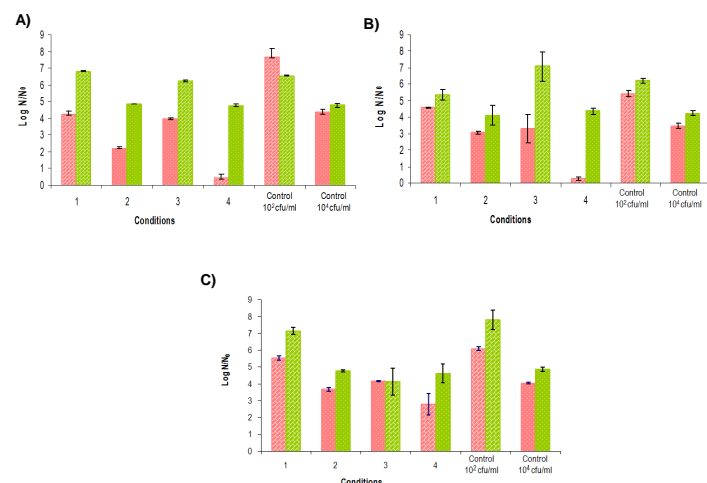


Fig. 2 Monitoring the number of viable cells in pasteurized milk: a) *L. monocytogenes*_1339 with *L. innocua*_11288; b) *L. monocytogenes*_1340 with *L. innocua*_11288; c) combination of all 6 strains of *L. monocytogenes* with *L. innocua*_2030c; ■ - *L. monocytogenes* (10^2 cfu/ml); ■ - *L. monocytogenes* (10^4 cfu/ml); ■ - *L. innocua* (10^2 cfu/ml); ■ - *L. innocua* (10^4 cfu/ml).

Except for condition 3, in all the mixtures, it was observed the inhibition of *L. monocytogenes* in the presence of *L. innocua* (Fig 2). However, it was more significant when *L. innocua* was presented in higher concentration (condition 4).

Previous studies only refer the inhibition of *L. monocytogenes* in the presence of *L. innocua* however, in this study, in the case of mixture C, it was also verified inhibition of *L. innocua* when *L. monocytogenes* was presented in higher concentration (condition 3).

► **Detection of inhibitory activity produced by *Listeria* strains:**

No inhibitory activity of *L. innocua* against *L. monocytogenes* or *L. monocytogenes* against *L. innocua* was observed in the spot-on-lawn assays. However many authors (Yokoyama et al. 2005; Besse et al. 2005) demonstrated that *Listeria* species may produce inhibitory compounds such as bacteriocins that are active against other *Listeria* isolates.



FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

