A secretome-based methodology may provide a better characterization of the virulence of *Listeria monocytogenes*: Preliminary results

Paula Cabrita a,b,c, Catarina Fonseca a, Regina Freitas a,b, Ricardo Carreira d, Jose Luis Capelo e, Maria João Trigo e, Ricardo Boavida Ferreira a,b, Luisa Brito a,d,*

a CMAA/Laboratory of Microbiology, Instituto Superior de Agronomia, Technical University of Lisbon, Tapada de Ajuda, 1349-017 Lisbon, Portugal
b Instituto de Tecnologia Química e Biológica, New University of Lisbon, Apartado 127, 2781-901 Oeiras, Portugal
c Faculdade de Ciências e Tecnologia/SEGME, New University of Lisbon, 2829-516 Caparica, Portugal
d ESPCFOC Group, Nutrition & Bacteriology Area, Analytical or Food Chemistry Department, Science Faculty, University of Vigo at Ourense Campus, 32004 Ourense, Spain

ABSTRACT

Four strains of *Listeria monocytogenes* with different levels of virulence were studied. Two strains were consistently evaluated as virulent (strain 3077) and of low virulence (strain 3093), whereas the other two strains (3006 and 3049) originated conflicting results in what the evaluation tests were concerned: both were shown to exhibit low virulence when evaluated by in vitro assays, but virulent when the analyses were performed under in vivo conditions.

To clarify the virulence potential of the selected strains, a proteomic approach was used after incubating *L. monocytogenes* cultures under conditions favoring the expression of virulence factors (minimal medium, at 37 °C). Bacterial proteins present in the liquid culture media were precipitated from late exponential phase cultures, fractionated by SDS-PAGE and identified by MALDI-TOF-MS.

Three virulence factors differentially expressed were detected: protein p60, listeriolysin O (LLO) and internalin C (InIC). Clustering analysis of the four *Listeria monocytogenes* strains based on their secretome profiles allowed their categorization in two groups: the virulent group, composed by strains 3077 and 3049, and the low virulence group, containing strains 3093 and 3006. The results presented in this work suggest that the virulent potential of a particular *L. monocytogenes* strain may be predicted from the levels of both listeriolysin O (LLO) and internalin C (InIC) present in its secretome when the bacterium is grown under conditions favoring the expression of virulence factors. Following validation of this proposal through the analysis of a large array of strains, this methodology exhibits a great potential to be developed into an accurate and rapid method to characterize *L. monocytogenes* strain virulence.

© 2010 Elsevier B.V. All rights reserved.

* Corresponding author. Tel.: +351 21 365 3433/3240; fax: +351 21 365 3195.
* Correspondences: sbtrenca@fc.up.pt (L. Brito).

0039-0140/ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.09.039