

PathogenCombat  
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**NEW CHALLENGES IN FOOD PRESERVATION**  
Processing • Safety • Sustainability  
11-13 NOVEMBER 2009 • BUDAPEST, HUNGARY

**Tackling foodborne pathogens through the understanding of their behaviour in the food chain**

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FACOLTA AGRARIA  
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**Within Pathogen Combat:**

- Improvement of methods for detection of pathogens in foods with shorter times for analysis, quantification, definition of the vitality and profiling of expression of virulence genes;
- Development of new methods for the study of the behavior of the pathogens in food and the influence of food components on their fitness.

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**WP 4 objectives**

**Development of culture-independent molecular methods suitable for the detection and identification of foodborne pathogens and for the study of the microbial ecology of foodstuffs**

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**Organisms considered**

- *Listeria monocytogenes*
- *Escherichia coli* STEC
- *Campylobacter jejuni*
- *Mycobacterium avium* subsp. *paratuberculosis*
- Hepatitis E virus

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***Listeria monocytogenes***

Systemic listeriosis in humans is a **severe disease that primarily affects the elderly, people with compromised immune systems, pregnant women and fetuses causing miscarriages, and newborn infants.** In the European Union (EU) Member States, **a significantly increasing trend in the notification rate of listeriosis cases in humans was observed between 2002 and 2006. This notification rate remained at the same level in 2007** according to the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the EU. The proportion of food samples exceeding the legal food safety criterion for *Listeria monocytogenes* in EU Member States was reported in this report to be highest in **ready-to-eat (RTE) fishery products**, followed by **RTE meat products and cheeses.**

Source: EFSA

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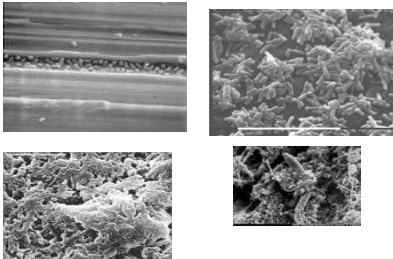
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***Listeria monocytogenes* forms biofilms !!!**



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**Listeria monocytogenes**

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)  
  
 International Journal of Food Microbiology 121 (2008) 99–105  
[www.elsevier.com/locate/jfoodmicro](http://www.elsevier.com/locate/jfoodmicro)

ELSEVIER

INTERNATIONAL JOURNAL OF  
Food Microbiology


Detection, quantification and vitality of *Listeria monocytogenes* in food as determined by quantitative PCR

Kalliopi Rantsiou<sup>a</sup>, Valentina Alessandria<sup>a</sup>, Rosalinda Urso<sup>b</sup>, Paola Dolci<sup>a</sup>, Luca Coccolin<sup>a,\*</sup>

<sup>a</sup> Dipartimento di Sperimentazione e Protezione delle Risorse Agroalimentari, Facoltà di Agraria, Università degli studi di Torino, Turin, Italy  
<sup>b</sup> Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università degli studi di Udine, Udine, Italy

Received 13 July 2007; accepted 9 November 2007

This protocol was subsequently used to check *L. monocytogenes* occurrence in artisanal products produced in the Piedmont region (Northwest Italy). Raw milk (3), curd (4), cheese (101) and sausage (50) were sampled. Six cheeses produced with raw milk resulted positive after enrichment, but were <math><10^3</math> cfu/g at  $t_0$  (manuscript in preparation)

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**Listeria monocytogenes**

Molecular methods to assess *Listeria monocytogenes* route of contamination in a dairy processing plant

Valentina Alessandria, Kalliopi Rantsiou, Paola Dolci, Luca Coccolin\*

Di.Va.P.R.A., Faculty of Agriculture, University of Turin, Italy

*IJFM, submitted*

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**Listeria monocytogenes**

Application of the qPCR method in a dairy company

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    graph TD
      A[Sample Cheese - Swab - Brine] --> B[+ Half Fraser]
      B --> C[Extraction of DNA]
      B --> D[Counts on Oxford agar]
      B --> E[Incubation at 37°C o/n]
      C --> F[Quantification by qPCR if at T24 positive]
      D --> G[Isolation of suspected colonies]
      E --> H[Extraction of DNA]
      E --> I[Streak on Oxford agar]
      G --> J[Confirmation of L. monocytogenes by PCR]
      H --> K[qPCR]
      I --> L[Isolation of suspected colonies]
      L --> M[Confirmation of L. monocytogenes by PCR]
    
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## Conclusions

"The results of this study highlight once more how it is **vital for food producing companies to have control of the contamination routes of foodborne pathogens in their plants**. This can be achieved with **the implementation of correct sampling schemes and adequate cleaning and disinfection procedures**. In addition, **the choice of the analytical procedure** should also be considered as a relevant decision. As demonstrated in this study, **traditional methods only partially can monitor the presence of *L. monocytogenes* in the processing plant**. Especially when **environmental conditions influence the fitness and behavior of the pathogen**, such as in the brines (with 15-20% salt) or in the **equipment swabs** (where cells have to respond to several stresses such as disinfectants, starvation and dried conditions), **molecular methods should be used to properly detect it**. It has been previously proven that *L. monocytogenes* undergoing stresses enters a **viable but not culturable state (VBNC)** that makes **impossible its recovery by culture dependent methods** (Rowan, 2004). The application of molecular methods can result in a better comprehension of the spread of a specific pathogen in a processing plant, thereby allowing the implementation of corrective actions to eliminate or decrease the risk associated with its presence in the final product."

*Alessandria et al. IJFM submitted*  
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### *In vitro* experiments in laboratory medium underline an heterogeneity of *L. monocytogenes* virulence expression !!!

	5	SCOTT A	162	70	NCTC 86	18	EGDE	19	V7	3	36	vba
stx2	0.142	0.247	0.332	0.447	0.297	0.095	1.007	1.027	1.517	2.262	3.222	<math>p</math><math>=0.001</math>
pta	0.035	0.207	0.027	0.257	0.127	1.007	0.127	0.007	0.337	0.137	0.137	<math>p</math><math>=0.001</math>
hly	0.02	0.03	0.48	0.47	0.27	0.63	1.00	0.24	1.77	0.59	0.43	ns
hly	0.02	0.07	0.21	0.04	0.45	0.77	1.00	0.07	0.35	0.31	0.18	ns

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### Expression analysis in real food samples

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**Future prospective**

**Profiling and expression of  
viability/stress response/  
adhesion /virulence by using  
customized microarrays**

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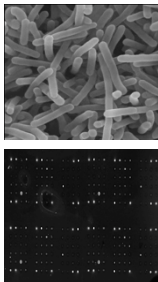
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**SureArray Listeria**

- Virulence array based on *Listeria monocytogenes* EGDe & F2365
- Expression analysis
- 7.5 x 2.5 cm Epoxy slide
- 72 unique probes
- Target list available on request
- 4 replicates of each probe
- 2 identical subarrays on each slide
- Open system: cDNA biotin-labeling, several options for staining, e.g. fluorescence
- Available for research



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**Silver staining**

- Removing of unspecific hybrids
- Pre-Blocking
- Incubation with Anti-biotin conjugate gold
- Washing and Rinsing
- **Staining:**  
Gold label catalyzes silver precipitation
- **Scanning:**  
Eppendorf silverquant scanner



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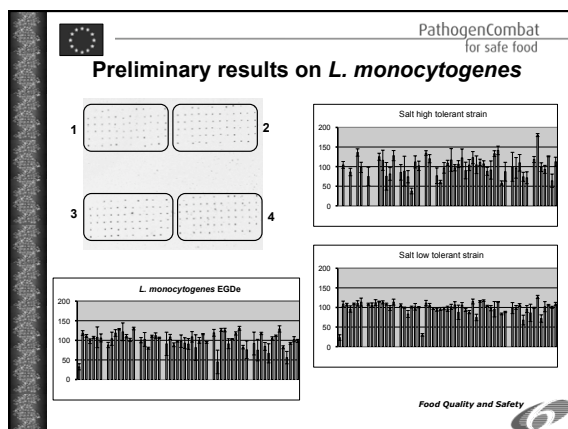
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### Acknowledgments

<p><b>WP4</b> UNIUD/UNITO Kalliopi Rantsiou Rosalinga Urso Paola Dolci Alessandria Valentina</p>	<p><b>Expression analysis</b></p> <p><b>WP2</b> LIFE Lene Jespersen Ingher Olesen Mogens Jakobsen</p> <p><b>WP7</b> LUND Peter Radstrom</p>	<p><b>qPCR development</b></p> <p><b>WP6</b> GENT Mieke Uyttendaele</p> <p>PROFOS Stefan Miller</p>	<p><b>Microarrays</b></p> <p><b>WP5</b> CONGEN Matthias Kuhn</p>
<p><b>NCTI and II</b> STUTT Susanne Braun Klaus Hädwiger</p>	<p><b>Data analysis</b></p> <p><b>WP14</b> AUA Maricos Mataragas Eleftheros Drosinos</p>	<p><b>Management</b> Mogens Jakobsen Vicki Lei Line Sanderbank Olesen Jorn Dalgaard Mikkelsen</p>	<p>Food Quality and Safety </p>

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