New techniques for sampling *Listeria monocytogenes* from food industry surfaces

S. Porta[1], M. S. Gião[2], L. Verdú[1], C. W. Keevil[2], J. Belenguer[1]
1 Ainia Centro Tecnológico, *Parque Tecnológico de Valencia, Paterna (Valencia) corresponding author: sporta@ainia.es; www.ainia.es*
2 University of Southampton, School of Biological Sciences, Southampton, UK

Introduction

Despite the adoption of tight control measures to ensure food safety in European countries, the prevalence of pathogens in food products is still responsible for several outbreaks. *Listeria monocytogenes* has become one of the main pathogens transmitted by food. The contamination of processed food is often caused through contact with contaminated surfaces, where bacteria can attach and rearrange in a complex structure called biofilm. The microbiological safety of the final product can be assessed by the detection of *L. monocytogenes* on food processing equipment and on surfaces where food might contact. The microbiological control of surfaces still relies on traditional methods (sponges, swabs, contact plates) that have some limitations related to the low recovery rate of microorganisms, mainly due to adhesion forces established between the microorganisms and the surfaces. A novel compressed air based method is used in this work as an alternative technique to sample and recover *L. monocytogenes* from industrial surfaces.

Materials and Methods

Stainless steel coupons (SS) were artificially contaminated with planktonic cells and biofilms in order to simulate industrial contamination of surfaces **(A)**. Later, compressed air was applied on the surface to remove cells **(B)**

(A) Simulation of industrial contamination

1. Selection of surfaces and *Listeria monocytogenes* strains

316 Grade SS coupons (5x5cm^2, 1x1cm^2) were sterilized and artificially contaminated with *L. monocytogenes* NCTC 11994 (surface inoculation with planktonic cells) and NCTC 13372 (for biofilm formation)

2. Inoculation of samples with planktonic cells

An overnight culture in BHI broth (concentration calculated by plating in agar 37ºC-24h) was used to inoculate the coupons using two different methods:

- disposable inoculation **spreader**
- **atomizer** (A4704 Airbrush, Aztek)

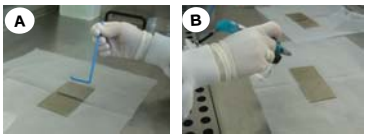

Fig 1. Inoculation of SS coupons with a spreader (A) and a atomizer (B)

3. Biofilm formation

A six-well plate system was used for biofilm formation on 1 cm^2 SS in BHI, TSB, TSBYE media and filtered tap water at 4, 22, 30 and 37ºC.

4. Microorganisms recovery

- Two methods were tested for planktonic cells: **Scraping** the surface with a palette and **sonication** in a diluent (5min). Recovered microorganisms were plated onto nutrient media to count cultivable microorganisms.

- After 4h, 24h and 48h biofilms were:

  ➢ Observed *in situ* by **EDIC/EF microscopy** after staining with fluorescence stain (Live/Dead BacLight kit).

  ➢ Recovered by scraping the coupon using glass beads. Total cells were quantified by staining them with SYTO 9. Cultivable cells were quantified by plating them onto nutrient media.

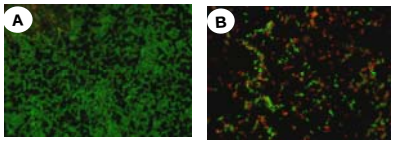

Fig 2. 24h Biofilm formed at 37ºC (A) and 22ºC (B), observed under EDIC/EF microscope.

(B) Testing contaminated surfaces with compressed air

1. A special **tip was designed** with a nozzle to focus air blown and a bacteriological filter to retain microorganisms after sampling (Fig. 5)

2. In order to maximize the impact of the injected air flow, **different parameters were tested on artificially contaminated samples** (with both planktonic cells and biofilms)- Table 1.

- Diameter hole of nozzles between 0.2 to 3.1 mm
- Different pressures (2-7 bars) and air flows (20-90 L/min)
- Different positions from the nozzle to surface (distance and angle)

3. After the tests, **remaining cells on surface were recovered** using recovery methods to measure the effectiveness of the new sampling technique.

Table 1. Parameters tests

Parameter	Test 1	Test 2	Test 3	Test 4	Test 5
P (bar)	2	2	7	7	7
Flow (L/min)	20	40	40	60	90
T (s)	60	60	60	60	60

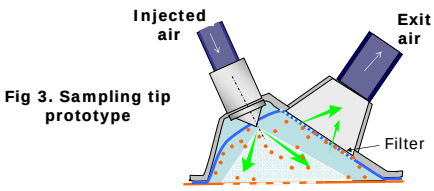

Fig 3. Sampling tip prototype

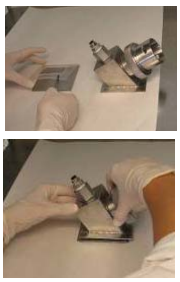

Fig 4. Compressed air testing

Results

(A) Simulation of industrial contamination

The best conditions to form *L. monocytogenes* biofilm were BHI medium at 22ºC for 24h. For planktonic cells, the best results were obtained using the atomizer as inoculation method and sonication as recovery method. Those methods and conditions were selected for compressed air sampling tests.

(B) Testing contaminated surfaces with compressed air

High levels of pressure show no effect on the viability of the microorganisms attached to surfaces. Best results were obtained with 7 bars pressure, 90 L/min, 3.1 mm diameter nozzle and a perpendicular position of the nozzle regarding to the tip. For distances higher than 15mm, nozzles were not effective.

Conclusions

- Results demonstrate that contamination on industrial surfaces can be efficiently simulated with the techniques developed in this work.
- Cells of *L. monocytogenes* attach to the substratum and form strongly adherent biofilms that require the application of a high pressure to ensure the detachment of food processing surfaces.
- Preliminary results suggest that compressed air could be an effective alternative method for sampling microorganisms from surfaces.
- Further optimization of application parameters as well as the development of a recovery method for sampling of *L. monocytogenes* are under study.

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