Development of a Mass Spectrometry Imaging (MSI) approach to explore Listeria monocytogenes biofilms exposed to a dehumidification stress

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Introduction

Human listeriosis cases are due to the ingestion of foods contaminated with *Listeria monocytogenes*. Control of this pathogen is a daily concern for many food industries and represents a major challenge in terms of hygiene and public health. *L. monocytogenes* is capable of forming biofilms and surviving harsh environmental conditions. It can persist in microniches despite the daily procedures of cleaning disinfection. These procedures also cause significant variations in the relative air humidity and the dehumidification of the premises is an important step for the control of microbial contaminations Despite this, part of the population is able to resist these treatments. The molecular mechanisms by which biofilms adapt to dehumidification, often applied empirically, are not well known and it is important to develop tools to better decipher them.

Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) is a surface-sampling technology that can determine spatial information and relative abundance of analytes directly from biological samples. Basically applied to animal or human tissues in biomedical research, applications have diversified in other scientific fields. By assimilating a microbial biofilm to a tissue, this approach has been implemented to investigate its potential to provide relevant data on molecular mechanisms that allow cells to adapt to air dehumidification stress (moderate desiccation).

This study aims to develop an IMS approach and an *in situ* identification method to explore the protein expression within *L. monocytogenes* biofilms exposed to air dehumidification.

Workflow

MALDI IMS approach

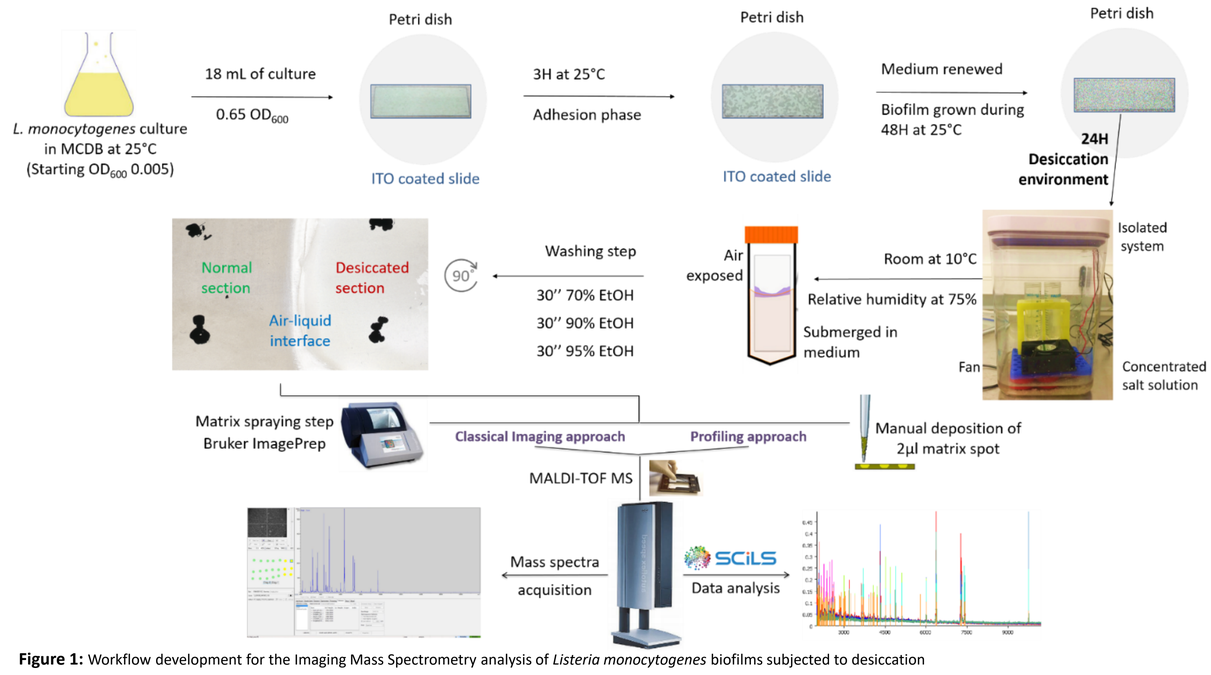


Figure 1: Workflow development for the Imaging Mass Spectrometry analysis of *Listeria monocytogenes* biofilms subjected to desiccation

In situ extraction of proteins and identification by nanoLC-MS/MS

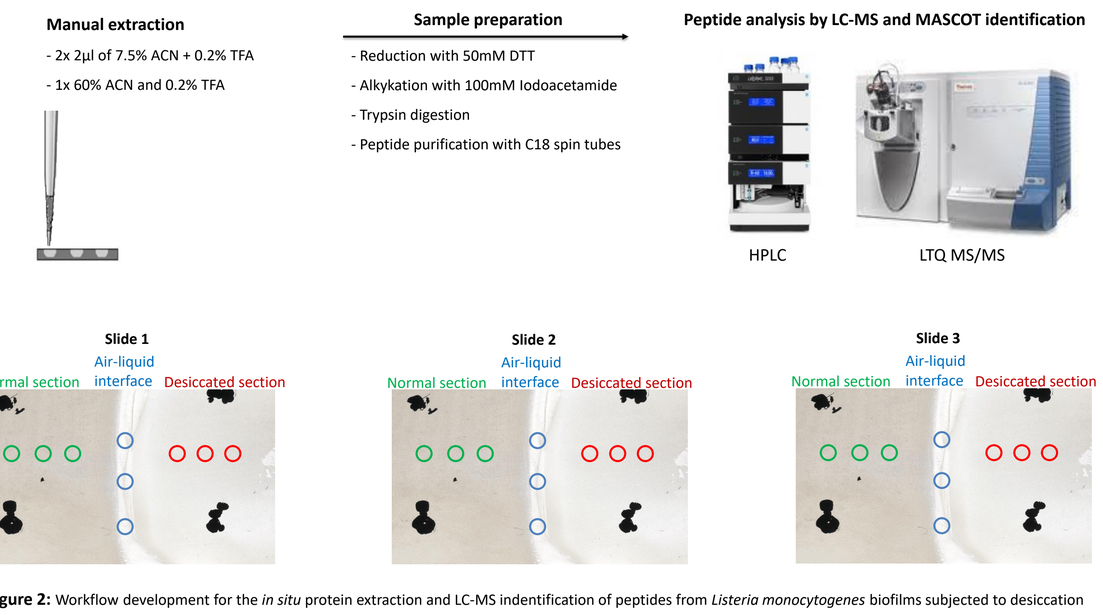


Figure 2: Workflow development for the *in situ* protein extraction and LC-MS indentification of peptides from *Listeria monocytogenes* biofilms subjected to desiccation

Results

Classical imaging approach

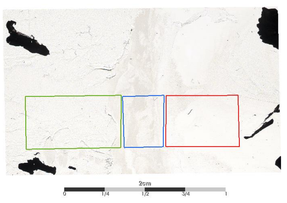


Figure 3: MS analysis across a biofilm section; Resolution: 100 µm; 500 shots per spot

Normal section
Air-liquid interface
Desiccated section

Profiling approach

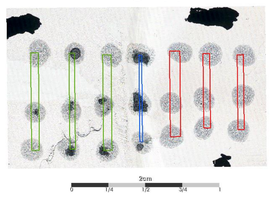
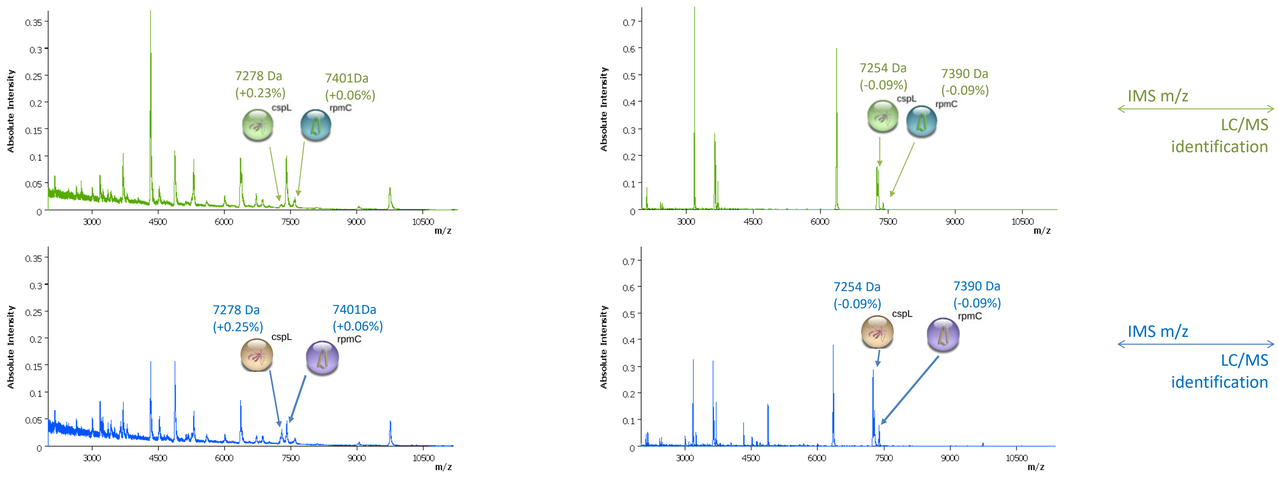


Figure 4: MS analysis in the drops of matrix; Resolution: 2000 µm; 4000 shots per spot



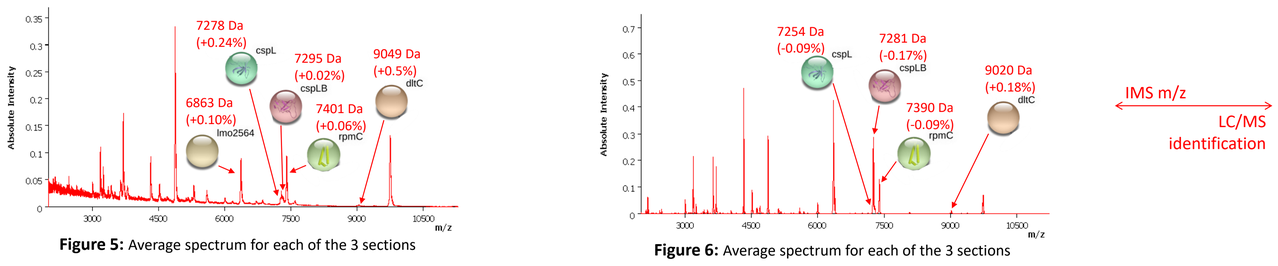
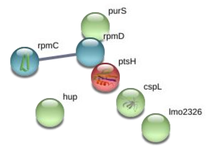


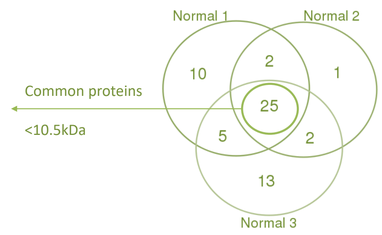
Figure 5: Average spectrum for each of the 3 sections

Figure 6: Average spectrum for each of the 3 sections

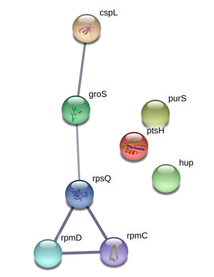
Figure 7: Venn diagrams for all identified proteins from each of the biological replicates (slide 1: 442 proteins; slide 2: 462 proteins; slide 3: 373 proteins)

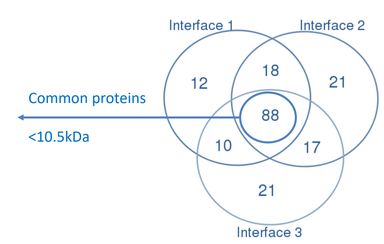
Entry name	Protein	Mass (Da)
RL30	50S ribosomal protein L30	6,493
CSPA	Cold shock-like protein CspLA (CspL)	7,266
RL29	50S ribosomal protein L29 (rmpC)	7,402
Q92AN7	Phosphoribosylformylglycinamidine PurS	9,360
PTHP	Phosphocarrier protein HPr	9,404
Q92A74	Hup protein	9,882
Q8Y4V4	Lmo2326	10,413





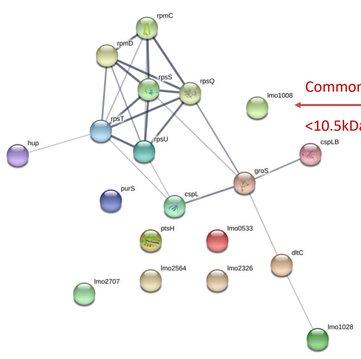
Entry name	Protein names	Mass (Da)
RL30	50S ribosomal protein L30	6,493
CSPA	Cold shock-like protein CspLA (CspL)	7,266
RL29	50S ribosomal protein L29	7,402
Q92AN7	Phosphoribosylformylglycinamidine PurS	9,360
PTHP	Phosphocarrier protein HPr	9,404
Q92A74	Hup protein	9,882
RS17	30S ribosomal protein S17	10,036
CH10	10 kDa chaperonin (GroES protein)	10,064





Entry name	Protein names	Mass (Da)
RL30	50S ribosomal protein L30	6,493
RS21	30S ribosomal protein S21	6,846
Y2564	Probable tautomerase	6,861
CSPA	Cold shock-like protein CspLA (CspL)	7,266
CSPB	Cold shock-like protein CspLB (CspB)	7,298
RL29	50S ribosomal protein L29	7,402
Y1028	UPF0356 protein lmo1028	8,292
Q8Y3X2	Lmo2707 protein	8,337
Q92D14	Lmo1008 protein	8,956
DLTC	D-alanine--poly(phosphoribitol)	9,010
RS20	30S ribosomal protein S20	9,169
Q92AN7	Phosphoribosylformylglycinamidine PurS	9,360
PTHP	Phosphocarrier protein HPr	9,404
Q92A74	Hup protein	9,882
Y533	UPF0237 protein lmo0533	9,943
RS17	30S ribosomal protein S17	10,036
CH10	10 kDa chaperonin (GroES protein)	10,064
Q8Y4V4	Lmo2326 protein	10,413
RS19	30S ribosomal protein S19	10,475

Table 1: Description of the proteins present in Figure 9; Highlighted proteins correspond to the ones correlated with m/z intervals from IMS and profiling (mass tolerance: 0.5%)



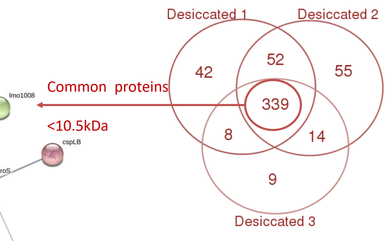


Figure 9: STRING protein-protein interaction with the common proteins identified, with mass below 10.5kDa, from each of the three section

Figure 8: Venn diagrams for all identified proteins by (Normal section: 58 proteins; Interface: 187 proteins; Desiccated section: 519 proteins)

Discussion

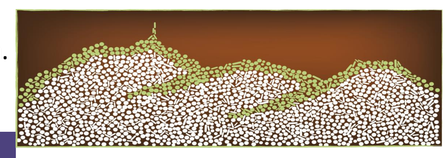
The 3 sections of analysis presented good spectra in both approaches, classical imaging and profiling. The profiling showed lower level of background noise and higher intensity of peaks than the classical approach. This is due to the higher ratio matrix-sample present in the profiling and also the superior signal accumulation obtained in one spot of analysis.

The *in situ* extraction of proteins resulted in a high and reproducible number of proteins identified. The higher amount of proteins identified in the interface and desiccated section could be related to their exposition to the stress condition, and therefore an increase of cell lysis and proteins available for extraction.

The back correlation between the significative m/z intervals and the *in situ* identification lead to 5 different proteins being assigned to the mass spectra (mass tolerance of 0.5%): In the normal section and interface, a cold shock protein (CspLA) and a 50S ribosomal protein L29; From the desiccated section, a second CspL (CspLB) which is involved in adaptation to atypical conditions, a tautomerase lmo2564 and the membrane-associated protein D-alanine-poly(phosphoribitol) ligase, which is involved in the pathway lipoteichoic acid biosynthesis. Loss of D-alanylation of lipoteichoic acids alters the cell surface charge and results in reduced biotic attachment and biofilm production.

Conclusions

These MALDI IMS and innovative protein identification approaches contributes to dissect the spatial proteome of an intact bacterial biofilm giving a new insight into protein expression relating to biofilm adaptation.



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