Adaptation of Listeria monocytogenes to temperature: exploration of intracellular subproteome through shotgun proteomics

Tiago Santos, Christophe Chambon, Didier Viala, Julia Esbelin, Michel Hébraud

Adaptation of *Listeria monocytogenes* to temperature: exploration of intracellular subproteome through shotgun proteomics

Tiago Santos[1], Christophe Chambon[2], Didier Viala[2], Julia Esbelin[1] and Michel Hébraud[1,2]

[1] Université Clermont Auvergne, INRA, UMR MEDiS, F-63122 Saint-Genès Champanelle, France
[2] INRA, Plateforme d'Exploration du Métabolisme (PFEM), F-63122 Saint-Genès Champanelle, France

Introduction

Listeria monocytogenes is a foodborne pathogen that can cause serious invasive human illness (listeriosis) in susceptible patients. Most human listeriosis cases appear to be caused by consumption of refrigerated ready-to-eat foods. Although initial contamination levels in foods are usually low, the ability of these bacteria to survive and multiply at low temperatures allows it to reach levels high enough to cause disease. It is able to grow in a broad spectrum of temperatures, between 1 and 45°C. **This study explores which cytoplasmic proteins (i) could be related to the adaptation of *L. monocytogenes* at different temperatures and (ii) are differently expressed according to the planktonic versus biofilm mode of growth.**

Materials and Methods

Protein extraction from cultures in planktonic mode of growth was performed in stationary phase after 48h, 20h and 16h at 10°, 25° and 37°C, respectively. For sessile cells (biofilms), protein extraction was carry out in mid-log phase after 24h, 8h and 4h at 10°, 25° and 37°C, respectively. In the cellular fractionation method (Figure 1) cells were washed twice in Tris-EDTA. Pellet was resuspended in 1 ml TE and bacterial cells were broken using a cell disrupter by applying 2.5 kBar pressure. Insoluble materials containing cell walls were removed by centrifugation. After trypsin digestion of proteins, peptides separation and identification were performed by shotgun proteomics (LC-QTOF-MS/MS) and MS data were analysed by Progenesis QI (Figure 2).

Results

The first preliminary results from cells in biofilm at mid-log phase and planktonic cells at stationary phase allowed to identify 845 and 954 proteins respectively. After analysis of variance, 14 proteins from the biofilm samples and 15 from planktonic samples were statistically significant (a p-value of <0.05 was considered significant). Many of the identified proteins are connected to basic cell functions (Figure 2 and 3) but some are related to the temperature adaptation (Table 1 and 2). Among the thermoregulated proteins, some are particularly overexpressed at 37°C, the temperature at which *L. monocytogenes* is virulent, and others at low temperature condition, as those prevailing in food workshops.

Fractionation and LC-MS/MS workflow

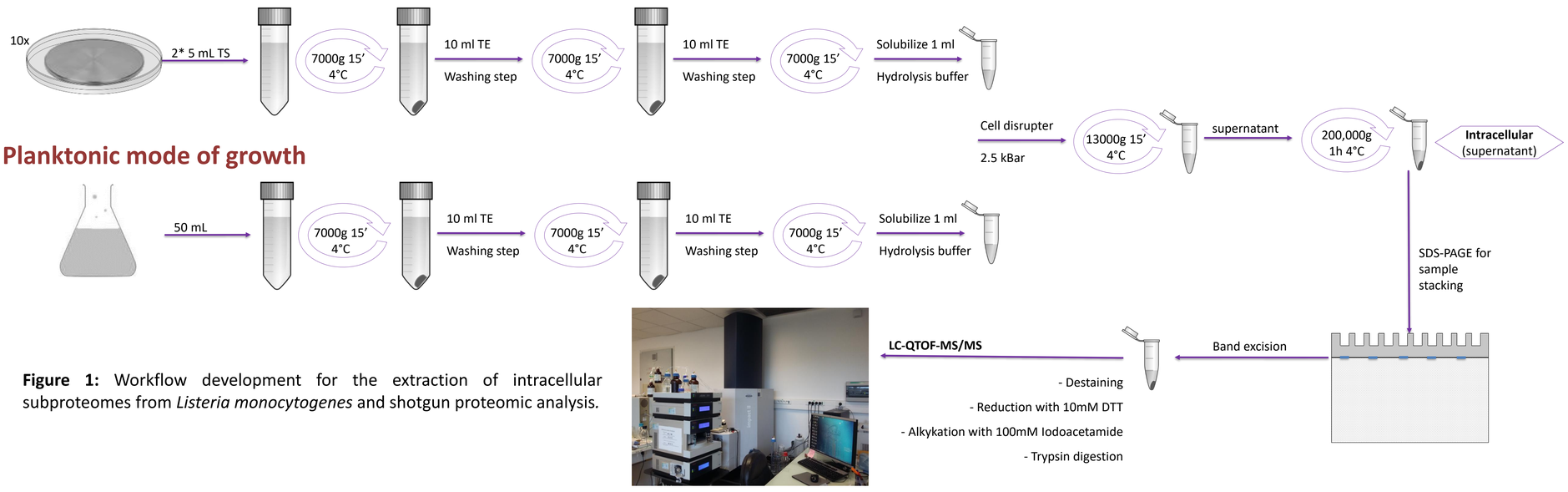

Figure 1: Workflow development for the extraction of intracellular subproteomes from *Listeria monocytogenes* and shotgun proteomic analysis.

Discussion

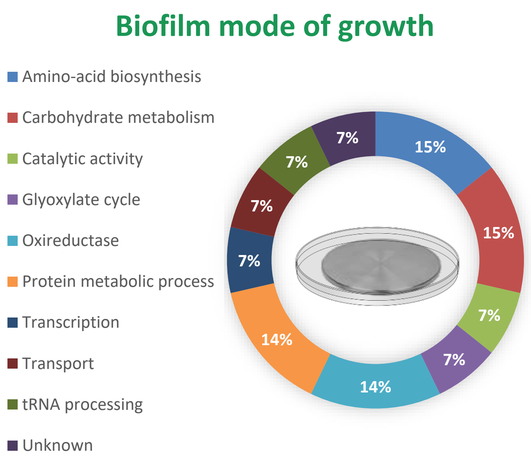

Figure 2: Distribution by biological process of the 14 statistical significant identified proteins from biofilm cultures and extraction at the mid-log phase.

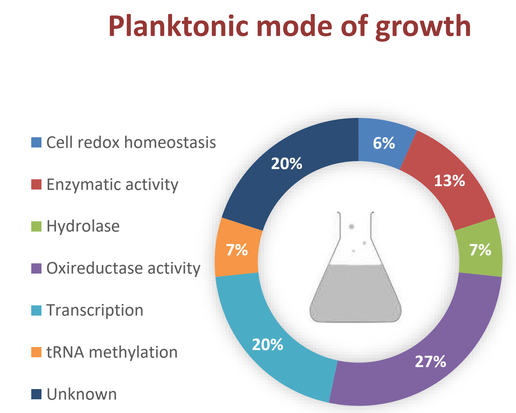

Figure 3: Distribution by biological process of the 15 statistical significant identified proteins from planktonic cultures and extraction at the stationary phase.

- The inosine-5'-monophosphate dehydrogenase guaB was identified with higher abundance at 37°C. The overexpression of guaB *in L. monocytogenes* could reflect a particular need for purines in surviving cells where DNA is being repaired.
- Lmo0135, which was also more abundant at 37°C, has a peptide transporter activity shown to be required for *L. monocytogenes* virulence and acid resistance.
- The ATP-dependent protease hslV is important for managing protein levels and directing stress responses and here it was detected in higher abundance in the 10°C condition.
- Glycerol-3-phosphate dehydrogenase glpD is known to be involved in glycerol uptake and metabolism, this oxireductase was more abundant in the 37°C extraction.

- FtsK protein is a cell division protein located at the cell septa and which coordinates chromosomal dimer segregation during cell division resulting in the separation of the daughter cells. Universal stress proteins (Usp) of *L. monocytogenes* are up regulated by σB. Usps are proteins that accumulate in cells during stationary phase and during a variety of stress conditions (heat shock, ultraviolet light, ethanol stress etc.) causing growth arrest in cells. Furthermore, Usp proteins are required for the management of DNA damage and are induced by mutations in the FtsK protein. In *L. monocytogenes*, FtsK expression is σB-dependent, suggesting coordinated expression with Usps to prevent chromosomal damage during cell separation. In this analysis, from stationary phase, this DNA translocase was more abundant at 37°C.

Table 1: Statistical significant identified proteins of interest from biofilm cultures and extraction at the mid-log phase.

Gene	Acession	Highest mean condition	Lowest mean condition	Protein	Function	Subcellular location
guaB	Q926Y9	Condition 37°C	Condition 25°C	Inosine-5'-monophosphate dehydrogenase	Catalytic activity playing a role in the regulation of cell growth	Cytoplasm
Lmo0135	Q8YAJ0	Condition 37°C	Condition 25°C	Lmo0135	Peptide transporter activity	Periplasmic space
HSLV	Q8Y7J9	Condition 10°C	Condition 25°C	ATP-dependent protease subunit	metal ion binding	Cytoplasm
glpD	Q8Y7I4	Condition 10°C	Condition 37°C	Glycerol-3-phosphate dehydrogenase	Oxireductase	Cytosol

Table 2: Statistical significant identified protein of interest from planktonic cultures and extraction at the stationary phase.

Gene	Accession	Highest mean condition	Lowest mean condition	Description	Function	Cellular location
ftsK	Q8Y7A3	Condition 37°C	Condition 25°C	DNA translocase	DNA binding	Cell membrane

Conclusions

More biological replicates and shotgun analyses of intracellular subproteomes will complement these preliminary results so that comparisons of protein expression throughout several conditions (temperatures, mode / phase of growth) feed databases and help to model regulatory circuitry that drive adaptation of *L. monocytogenes* to harsh conditions. An ongoing surfaceome study in these different conditions will also contribute for these goal.

SMMAP 2017 2-5 Octobre 2017 Disneyland Paris

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement n°641984