Adaptive response of Listeria monocytogenes biofilms to a dehumidification stress

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Introduction

Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various types of surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments. Our study was focused on the adaptation of *L. monocytogenes* biofilms in response to desiccation stress mimicking food workshop conditions.

Objective: decipher the molecular mechanisms that allow adaptation of sessile cells to dehumidification stress mimicking food workshops conditions.

Methods

L. monocytogenes EGD-e and L028 biofilms were grown on stainless steel discs at 25°C during 24 h, pre-adapted to 10°C for an additional 24 h and placed in a ventilated desiccation chamber where the Relative Humidity (RH) was stabilized with saturated NaCl solution to obtain a RH of 75%.

Culture in **TSB** medium at 25°C	Cells transfer in **TSB 1/5** medium	Stainless steel discs inoculation **3 h at 25°C**	Elimination of planctonic cells and medium renewal **TSB 1/5, 24 h, 25°C**	Medium renewal **TSB 1/5 24 h, 10°C**
Inoculum preparation		**Adhesion**	**Biofilm growth**	**Cold adaptation**

Fig. 1: Optimized and standardized protocol to obtain desiccated biofilms

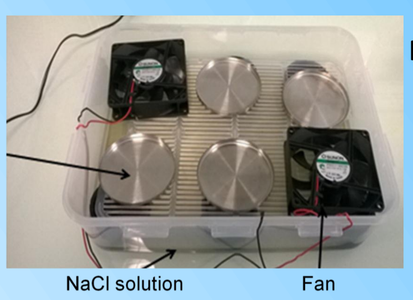

Fig. 2: Desiccation chamber

The intracellular and cell envelope (surfaceome) subproteomes were analyzed by shotgun proteomics (LC-MS/MS, OrbiTrap) and compared before, 3 h and 24 h after application of dehumidification stress. The surfaceome was studied through three complementary extraction methodologies (Fig. 3) by using (i) biotinylation of surface-exposed proteins with Sulfo-NHS-SS-Biotin and separation of labelled proteins by affinity chromatography with neutravidin from whole-cell lysates, (ii) enzymatic shaving of intact cells with trypsin to hydrolyze and release surface-exposed peptides and (iii) classical cell fractionation to recover membranes and cell wall proteins. Identified proteins were classified into three groups: surface, extracellular and cytoplasmic proteins according to the prediction of their subcellular location (Renier *et al*., 2012, PLoS One, 7: e42982). .

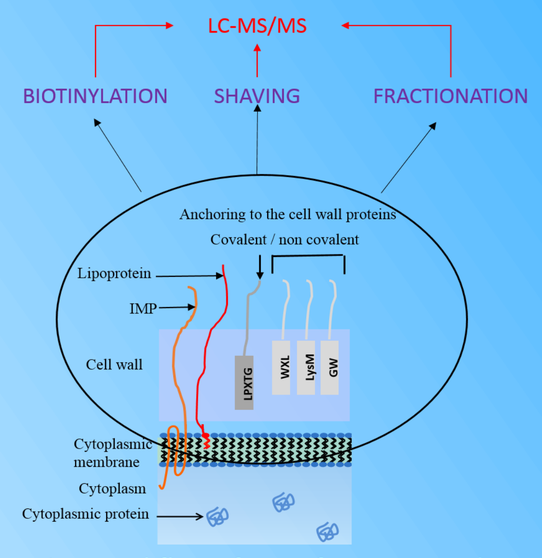

Fig. 3: Extraction workflows for surfaceome analyses

Results and Discussion

1. Intracellular analyses of dehumidified biofilms

Comparative analyses by LC-MS/MS of EGD-e and L028 *L. monocytogenes* intracellular subproteomes in control and dehumidified biofilms revealed that 38 and 65 proteins displayed significant differences of expression among the 628 and 420 identified proteins, respectively. 87% and 82% of differentially expressed proteins were predicted as intracellular, respectively. Most of them belong to information pathway and intermediary metabolism categories (Fig. 4). Surprisingly, few of them were common to the two strains (Fig. 4).

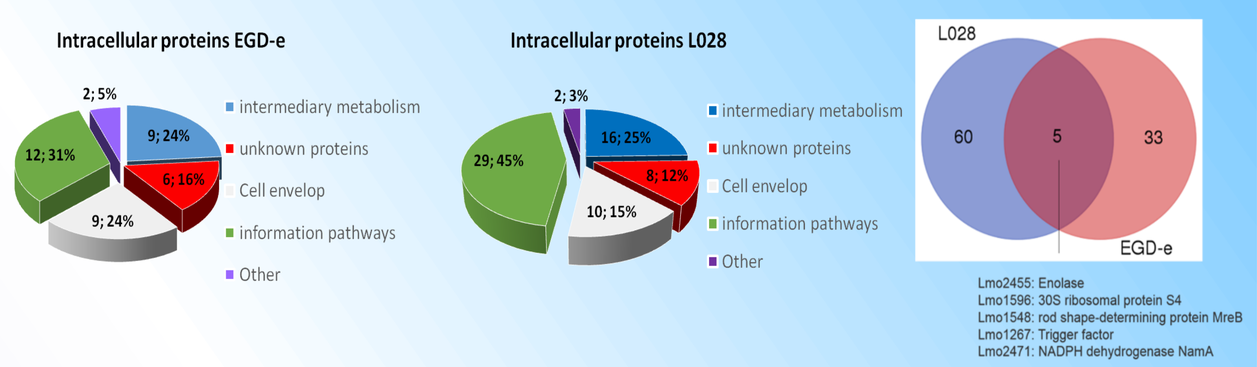

Fig. 4: classification of intracellular proteins differentially expressed and Venn diagram comparing these proteins between the two strains

2. Surfaceome analyses of dehumidified biofilms

The analyses of the surfaceome by the three complementary methods allowed to identify 21 and 28 proteins, predicted as cell surface proteins, differentially expressed during the dehumidification stress in EGD-e and L028 respectively. Most of these proteins were obtained by classical fractionation technique (Fig. 5).

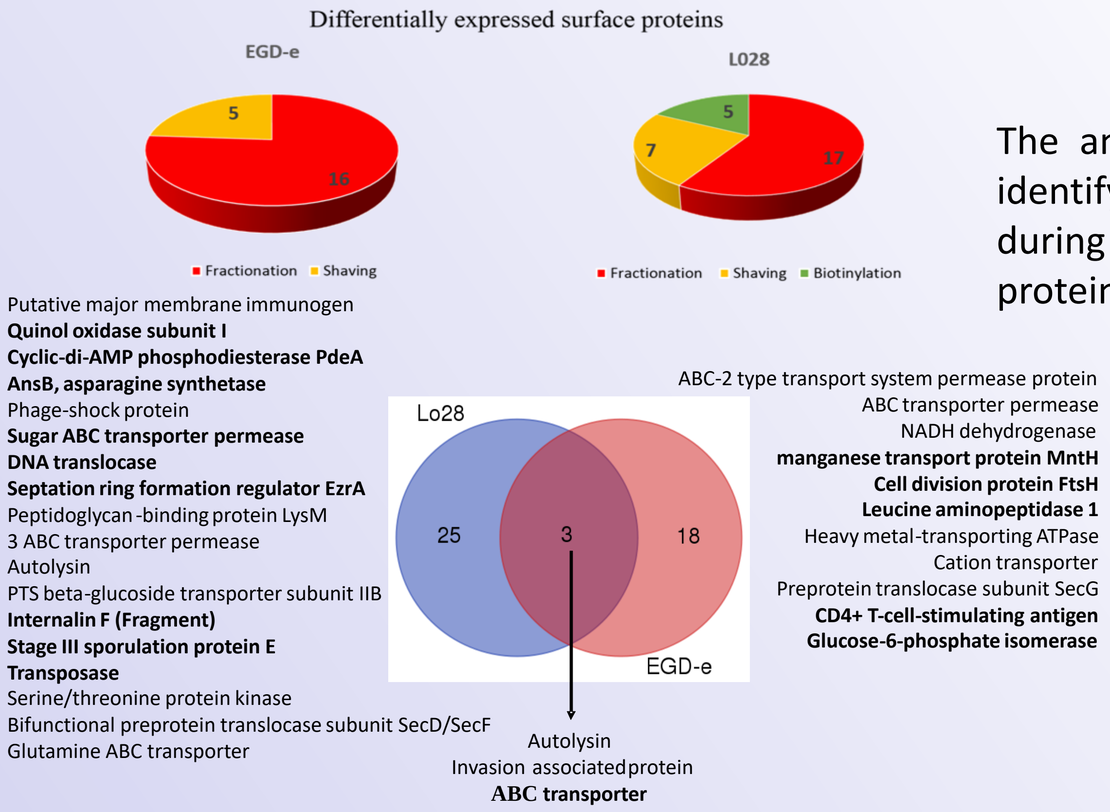

Fig. 5: Differentially expressed proteins identified by mass spectrometry and Venn diagram comparing these proteins between the two strains.

As for the intracellular proteins, it was surprising to find only three common proteins between the two strains. Two of them were underexpressed (an autolysin involved in peptidoglycan hydrolysis and Iap involved in the escape of *Listeria* cells to macrophages) and one overexpressed (ABC transporter). Among the other overexpressed proteins at 75% RH and potentially involved in adaptation and/or resistance to stress, it can be noticed for example the EzrA regulator, involved in cell division and essential in high pressure stress resistance, the "CD4 + T-cell-stimulating antigen", an overexpressed lipoprotein during a prolonged heat shock and a "sugar ABC transporter permease" (Fig. 5).

Conclusion

The variations of RH are very common events in the natural environments but also in food processing workshops consecutively to daily cleaning disinfection procedures. The mechanisms of adaptation to these stresses are poorly described in the litterature. Our first results from subproteomic analyses show that essential functions seem to be affected by RH decrease, but surprisingly, few proteins appeared common to the two *L. monocytogenes* strains. Moreover, the viable and cultivable population after 24 h at 75% RH was significantly reduced by 1.2 and 0.9 log for EGD-e and L028 respectively, which has consequences for proteomic analysis that must be taken into account. However, these first results need to be completed and deepened to better understand the physiological and molecular events involved, with the goal to improve the effectiveness of current cleaning disinfection procedures, while reducing their environmental impact.