Biofilms of Listeria monocytogenes submitted to a decrease in air relative humidity

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Introduction

Listeria monocytogenes is a foodborne pathogen associated with a high mortality rate in case of contamination. It is one of the major biological concerns in food hygiene for numerous food processing and conditioning industries. It may grow at refrigeration temperatures, in a wide range of pH, in salt concentrations up to 10% NaCl and low water activity. It is able to contaminate and persist in the food-processing environments, particularly through its capability to adhere and form biofilms on various surfaces. 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 and disinfection (CD) treatments. Air drying after CD procedures allows to limit microbial growth on solid surface and even affect the viability of microorganisms but its actual impact, as a function of the decrease in the air relative humidity (RH) reached, is not really known, nor the ability of bacteria adaptation to this treatment.

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 RH was stabilized with saturated NaCl solution to obtain a RH of 75%.

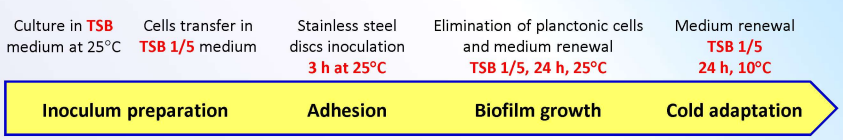
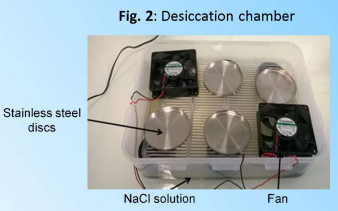


Fig. 1: Optimized and standardized protocol to obtain desiccated biofilms

Fig. 2: Desiccation chamber



The viability and different subproteomes were analyzed after 3 h and 24 h dehumidification by comparison with non-stressed biofilms. The subproteomes were compared by shotgun proteomics through three complementary extraction methodologies (enzymatic shaving, biotinylation and cell fractionation).

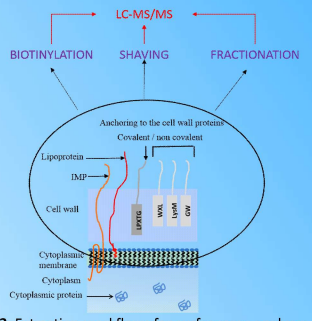
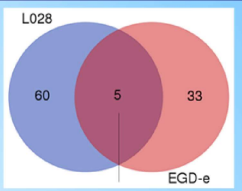


Fig. 3: Extraction workflows for surfaceome analyses

Results and Discussion

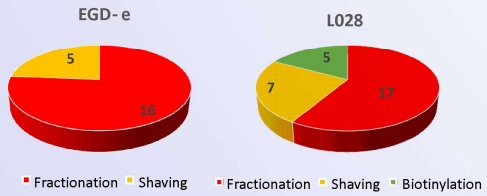
1. Intracellular proteome analyses of dehumidified biofilms

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. Surprisingly, few of them were common to the two strains (Fig. 4).



Lmo2455: Enolase
Lmo1596: 30S ribosomal protein S4
Lmo1548: rod shape-determining protein MreB
Lmo1267: Trigger factor
Lmo2471: NADPH dehydrogenase NamA

Fig. 4: Venn diagram comparing intracellular proteins differentially expressed 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 29 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).

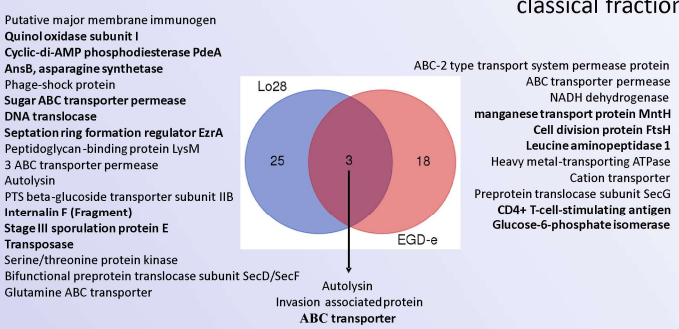


Fig. 5: Differentially expressed proteins identified by mass spectrometry and Venn diagram comparing these proteins between the two strains.

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 *Listeria 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. These results contribute to a better comprehension of mechanisms involved in the resistance and persistence of this pathogen in food plants despite the daily hygiene procedures.