STUDY OF *LISTERIA INNOCUA* HEAT RESISTANCE AFTER SUBLETHAL HEAT TREATMENT WITH MALDI-TOF

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INTRODUCTION

Stress adaptation causes changes in protein expression, which are reflected in the proteome of the microorganisms. For this reason, investigating the proteome of the microorganisms under various stress conditions can give illustrative thoughts about stress adaptation of the microorganisms. Since it is much faster than two-dimensional gel electrophoresis (2D GE) and able to catch low molecular weight stress proteins, Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) protein profiling also makes it possible to detect stress responses. Ribosomal and cell structure proteins show up as peaks in MALDI-TOF mass spectra. The changes in the characteristics of the peaks enable us to analyse the stress response of the microorganisms.

In this study, stress response of *Listeria innocua* in proteomic level was analyzed by cluster analysis of mass spectra obtained from MALDI-TOF for sublethal heat treated (46 °C for 30 min) and control samples. Additionally, $D_{60°C}$ values of both pre-treated and control samples were calculated by traditional culturing methods to investigate survival characteristics of the microorganisms.

PURPOSE

The overall objective of this study was to investigate the enhanced heat tolerance of *L. innocua* as a surrogate of *L. monocytogenes* after sub-lethal heat exposure. The aim of the current work focused on the survival characteristics and changes in the proteome of the bacterium.

MATERIALS AND METHODS

Listeria innocua strain (T1), a strain from the collection of MATE, was inoculated into Trypto-Casein Soy Broth (TSB, Biokar, France) (pH 7.3) at 37 °C to yield a cell population of approximately 8 lg CFU/mL.

Sample preparation for cluster analysis of MALDI-TOF peaks was made with modifications to the previous work of Schott et al. (2016). In order to obtain mass spectra, samples were taken from a water bath (Haake, Germany) every 3 minutes for 15 minutes for both control and sub-lethal heat exposed samples for 46 °C for 30 min. Mass spectra of the samples for mass range 2–20 kDa is obtained in MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) by 280 accumulated laser shots. All experiments were carried out in triplicates on different days.

Peak-based cluster analysis was applied to these pre-processed mass spectra to illustrate the stress response dynamics. Visualization by dendrogram was carried out with the KNIME Analytics Platform (Version 4.2.1) with R Foundation for Statistical Computing (Vienna, Austria). A package for RStudio software, adegenet was used for discriminant analysis of principal components (DAPC) to analyse the differentiation of stress responses (Jombart, 2008). In the end, DAPC maintains a barplot of eigenvalues and a scatterplot representing individuals as dots and groups as inertia ellipses.

The $D_{60°C}$ -values of the strain after the sub-lethal heat exposure of 30 min at 46 °C with the control samples were calculated to determine whether the sub-lethal heat exposure increased the D-value as previously described by Farber and Brown (1990).

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RESULTS AND DISCUSSION

Thirty-four mass spectra from control samples and sub-lethal heat exposed samples at 46 °C for 30 min were analysed to check possible differentiation in the proteome level. These thirty-four samples were grouped into three different clusters (Figure 1). This separation lies in the mathematical approach, discriminant analysis of principal components (DAPC).

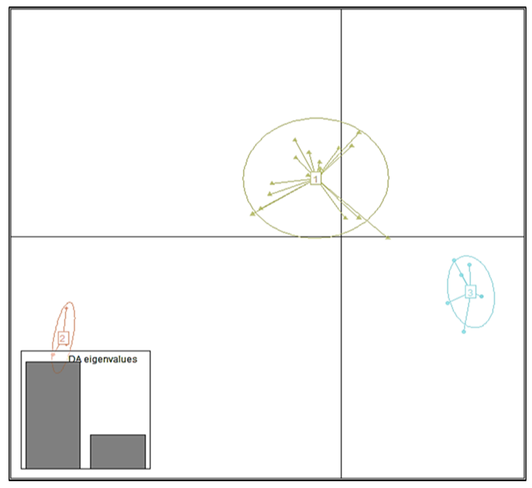

Fig 1. Clusters of the MALDI-TOF MS peaks, obtained from the DAPC analysis of the peaks from control and pre-heat exposed samples at 46 °C to 30 min.

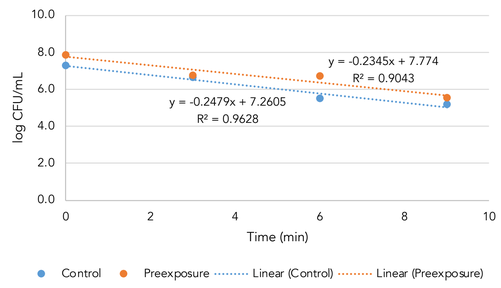

Fig 2. Enhanced heat resistance of *L. innocua* T1 at 60 °C when pre-exposed for 30 min at 46 °C.

Table 1. Effect of pre-exposure to sub-lethal temperatures of 46 °C for 30 min on the $D_{60°C}$ values for *Listeria innocua* T1 in Tryptic Soy Broth

Exposure time to sub-lethal temperature (min)	$D_{60°C}$ values ($\pm Standard\ Deviation$) (min)	
	Control	Pre-exposured
30	4.03 (±1.06)[a]	4.26 (±0.36)[a]
60	3.66 (±0.47)[a]	5.71 (±0.85)[b]

[a-b] For each row, different superscripts denote statistically significant

Seven of the control samples were in the first cluster; two were in the second cluster, and seven were in the third cluster. For the sub-lethal heat-treated samples, five were in the first cluster; two were in the second cluster, eleven were in the third cluster (data not shown). Overall, there was no meaningful separation of the samples into the different groups.

The inactivation kinetics of the *L. innocua* T1 after the sub-lethal heat exposure for 30 min and without prior exposure (control) are shown in Figure 2. Table 1 shows $D_{60°C}$ values of sub-lethal heat exposed samples at 46 °C for 30 min and non-prior heat exposed (control) samples. There was no significant difference in $D_{60°C}$ values between the samples after prior exposure of 46 °C for 30 min and the control ($p > 0.05$).

The hypothesis was that after the changes in the proteome of the samples, control and pre-heat exposed samples may differ in different clusters. Alternatively, samples could be separated from each other after a particular exposure time, so it can be proven that there is a specific time needed for the changes in the proteome of the samples. However, the separation of the samples did not give a revealing result. Three clusters were not meaningful, proving our results that there was no significant difference ($p > 0.05$) in the $D_{60°C}$ values of sublethal heat-treated samples for 46 °C for 30 minutes and control samples. According to our knowledge, this is the first research on peak based cluster analysis of heat stressed samples of *L. innocua* or *L. monocytogenes*.

CONCLUSION

Enhanced heat resistance of the *L. innocua* T1 as a surrogate of *L. monocytogenes* was investigated. No meaningful differentiation was found after analysing MALDI-TOF MS spectra of control and sublethal heat exposed samples at 46 °C for 30 min. Additionally, there was no significant difference in $D_{60°C}$ values between control samples and sublethal heat exposed samples at 46 °C for 30 minutes ($p > 0.05$).

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