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Transcriptional and physiological signs of *Listeria monocytogenes* adaptation to mild acid stress: seeking for biomarkers of acquired robustness

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Introduction:

Control of pathogens development in food or their inactivation through sanitising in food processing plants are often carried out with the use of organic acids. Time and acidity level of treatments are experimentally determined for each pathogen, and thus applied in food industries with the certainty of the biological hazard inactivation. However, the invasive pathogen *Listeria monocytogenes* is extremely versatile and can successfully adapt its physiology in mild-stress conditions, becoming subsequently resistant to more intense stresses, of the same or different type.

Purpose:

The aim of this study was to shed light on the long-term acid adaptation (LTAA) of the *Listeria monocytogenes* strain 10403S (Lm) during growth in planktonic condition.

Methods:

Lm was grown in presence of citric acid at mild-acidic conditions (pH 5.5 and 5.2) and in a neutral substrate. Furthermore, cold temperature (10°C) and anaerobiosis, have been included as additional variables in the LTAA. The acquired robustness was verified at various phases of growth by performing a sub-lethal shock at pH value 2 for 30 minutes. Samples collected during the three LTAA were subjected to microbiological and chemical analyses. Transcriptomic analysis was conducted for the LTAA experiments performed in absence of additional variables.

Results:

Anaerobiosis boosted the Lm growth and robustness, while at low temperature no differences in robustness have been observed between neutral and mild-acid conditions. Cells grown in mild acidity without additional variables showed an increased robustness to the shock compared to neutral conditions, which however was physiologically evident only once the stationary phase was reached. The RNA

sequencing analysis showed a clear involvement of the glutamate decarboxylase system in the acquisition of robustness, which was accompanied by a deregulation in sugars transport and consumption.

Significance:

This study highlighted the crucial importance of identifying transcriptional biomarkers that might predict the acquired resistance of *Listeria monocytogenes* to acid conditions.

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