Complementary analytical approaches improving knowledge on lactic acid bacteria cryoresistance.

A. Girardeau[1,*], J. Meneghel[2], S. Passot[1], I.C. Trelea[1], F. Fonseca[1]

[1] UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, Thiverval-Grignon, France
[2] Asymptote Ltd., General Electric Healthcare, Cambridge, UK

Freezing is the most used bacterial cell preservation technique yet still a process that can be damaging and lead to cell death. Cryosensitivity greatly varies depending on considered species or strains. Fourier Transform Infra-Red (FTIR) spectroscopy is a powerful technique allowing biochemical characterization of major cellular components (lipids, proteins, polysaccharides). However, the exploitation of bacterial spectral features under native aqueous environments is challenging due to the strong absorption of water in the mid-IR region. The identification of cryoresistance markers has thus so far been mainly done using FTIR spectra of dried cells. In this study, three lactic acid bacteria strains were selected for their contrasting cryo-sensitivities. The objective was to combine two complementary approaches: a dynamic approach that measures evolution of peak positions in the lipid region of IR spectra as a function of temperature, and a novel approach using a FTIR microscope enabling higher spatial resolution of cells (~10^3 to 10^4 cells) in an aqueous environment. The most cryoresistant strain displayed a strikingly different membrane lipid phase transition compared to the other two: although phase transition happened very abruptly and at a low temperature (-14 °C), in gel phase, lipid membranes of the resistant strain maintained a higher degree of disorganization. These observations could be attributed to high unsaturated fatty acid content (>66% of C18:1). In addition to confirming the lipid membrane's role in cryoresistance, this study has brought to light the potential role of other cellular components by removing the spectral contribution of water in spectra obtained with the FTIR microscope. This was achieved using a specially developed Matlab program, providing access to a large spectral region encompassing both the protein and carbohydrate regions (1800–975 cm^{-1}). Good discrimination and visualization of population heterogeneity was observed, indicating that markers outside the lipid region must also contribute to cryoresistance.

*Corresponding author.
Tel.: +33 1 30 81 45 30
E-mail address: amelie.girardeau@inra.fr