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## Mapping membrane fluidity in single bacterial cells to study the impact of cryopreservation and its related stresses

Julie MENEGHEL<sup>a,b</sup>, Stéphanie PASSOT<sup>a</sup>, Stéphanie CENARD<sup>a</sup>, Matthieu REFREGIERS<sup>b</sup>, Frédéric JAMME<sup>b</sup>, Fernanda FONSECA<sup>a</sup>

<sup>a</sup> INRA-AgroParisTech, UMR 782 GMPA, F-78850 Thiverval-Grignon  
<sup>b</sup> Synchrotron SOLEIL, L'Orme des Merisiers, BP48, F-91191 Gif-sur-Yvette

[julie.meneghel@grignon.inra.com](mailto:julie.meneghel@grignon.inra.com)

Cryopreservation of bacteria leads to cellular damages and death. The bacterial membrane is primarily exposed to changing environments and it is believed to play a major role in freeze-resistance. Two physiological states of the lactic acid bacterium (LAB) *Lactobacillus delbrueckii* subsp. *bulgaricus* were generated: a freeze-resistant and a freeze-sensitive state (Gautier et al. 2013). They were associated with membranes exhibiting different compositions in fatty acyl residues and phase transition temperatures (Gautier et al. 2013). A dynamic measurement of membrane fluidity by fluorescence anisotropy upon cooling in single-cells using Synchrotron UV microscopy revealed that freeze-resistant cells were characterized by a higher degree of fluidity at any temperature, especially around zero degrees Celsius as water nucleates. Mapping anisotropy in individual cells also revealed a heterogeneous distribution in freeze-sensitive cells, with the presence of islets of high rigidity (Passot et al., 2014).

We further demonstrated that hyperosmolarity is the main stress suffered LAB during cryopreservation as the matrix cryoconcentrates (Fonseca et al., 2006; Meneghel et al., under review). Aiming at determining the impacts of hyperosmotic environments on the membrane of LAB fluidity, fluorescence anisotropy was measured at the population scale by spectrofluorimetry and at single cell scale by Synchrotron UV microscopy. Both approaches gave complementary information, but only the Synchrotron UV microscopy allowed the visualization of high rigidity domains appearing under hypertonic conditions in the freeze-sensitive cell.

Cellular mapping of membrane fluidity gives thus essential information for a better understanding of cryopreservation-related damages. Modifying LAB growth conditions could enable the adjustment of membrane composition and its subsequent biophysical properties in order to improve LAB freeze-resistance.

### References:

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