Lipid cell structure imaging and membrane fluidity measurement in single living microorganisms

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Lipid cell structure imaging and membrane fluidity measurement in single living microorganisms

▶ SCIENTISTS INVOLVED

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▶ REFERENCES

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▶ KEYWORDS

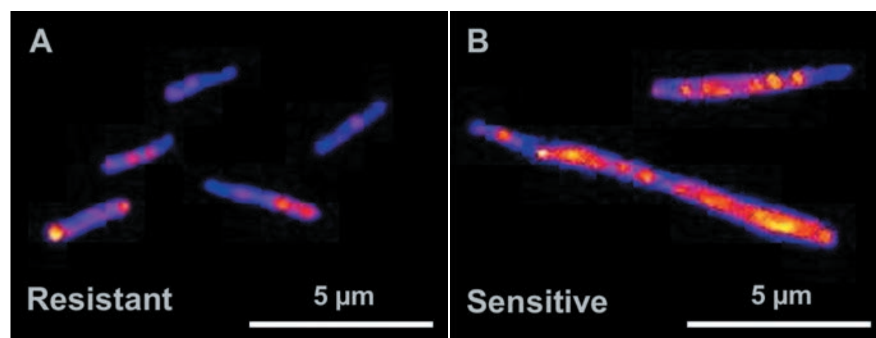
Membrane fluidity dynamics, anisotropy measurements, microorganisms, cell lipid structures.

The first interaction of cells with their environment is through plasma membrane. It is of great interest for numerous scientific teams to understand how this lipid structure is able to adapt to constraints, such as cold. One of the issues is to know the influence of plasma membrane fluidity and composition on the microorganism resistance to extreme environmental conditions such as freezing procedures or arctic climate.

▶ A PIONEER WORK USING TMA-DPH ON LACTIC ACID BACTERIA

The plasma membrane probes trimethylammonium-diphenylhexatriene (TMA-DPH) and DPH are commonly used to measure fluorescence anisotropy which makes possible the assessment of cell membrane fluidity, but few experimental devices are available to measure membrane fluidity at subcellular level. An original experimental device using fluorescence imaging to obtain membrane fluidity mapping of bacteria following cooling was developed in collaboration with the DISCO beamline.

Cryopreservation of bacteria requires specific cells pre-adaptation as well as a controlled freezing protocol to minimize membrane damage resulting from osmotic cell dehydration. Membrane fluidity has an important role in biophysical events taking place at subzero temperatures by facilitating or not the exchanges between intracellular and extracellular media. The degrees of liberty of TMA-DPH inside the bilayer provide a direct marker of membrane fluidity under DUV excitation. Microscope was modified to carry out fluorescence anisotropy measurement by inserting polarizers into the excitation and emission paths. Fluorescence polarization images of lactic acid bacteria were acquired at controlled temperatures from



▶ *Figure 1: Steady state fluorescence anisotropy images of individual bacteria observed at 4°C using Synchrotron DUV microscopy: freeze-resistant cells grown in MRS broth (A) and freeze-sensitive cells grown in mild whey medium (B). Yellow regions correspond to the highest value of anisotropy and blue regions to the lowest value of anisotropy (adapted from Passot et al. [1]).*

0°C to 40°C and anisotropy mapping of cell membrane at the subcellular level was obtained (fig. 1). The higher the anisotropy, the more rigid the membrane is.

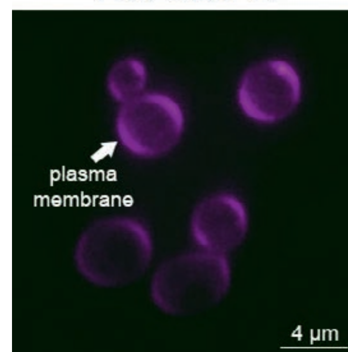
Results indicate that the membrane of freeze-sensitive cells rigidifies more extensively at low temperatures than freeze-resistant cells, and that intercellular and intracellular heterogeneities with subdomains concomitantly appear more frequently for freeze-sensitive cells (Fig. 1). The observation of the same feature at high osmolarity during a separate study of the effects of cold and osmotic stresses confirmed the negative impact of osmotic stress during freezing. The results give a glimpse of key levers for commercial production of interesting strains of lactic acid bacteria remained until now underexplored.

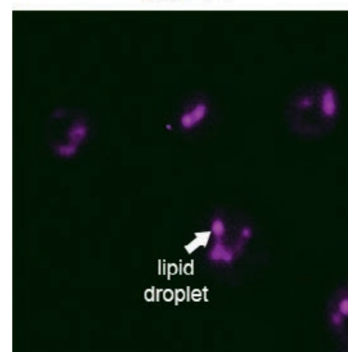
▸ NEW QUESTIONS AND TECHNICAL IMPROVEMENT: FROM PROKARYOTE TO EUKARYOTE

Interestingly, lipids and membrane dynamics is a common subject to many DISCO users and it became evident that users can join their expertise for efficient improvement of beamline devices and protocols. INRA scientists decided to combine their efforts through common proposals for measurement of cell plasma membrane fluidity in eukaryotic cells such as yeasts. This approach led to the great upgrade of the end-station (motorization of polarizers, homogenization of light) and establishment of protocols for reliable analysis of membrane fluidity in living eukaryotic cells with intracellular membrane compartments. Contrasted staining with TMA-DPH and DPH in living cells was observed. TMA-DPH stains mainly plasma membranes whereas DPH is rapidly internalized and stains the lipid droplets (Fig. 2). The use of DUV microscopy shows that TMA-DPH is better than DPH for plasma membrane fluidity measurement in eukaryotic living cells. Reinforced by these results we are now exploring the link between cell fatty acid composition (omega 3 content) and membrane fluidity in yeasts of interest for the food and non-food biotechnologies.

▸ ON THE WAY TO MULTI-MODAL IMAGING

The INRA consortium is currently working on lipid imaging at SOLEIL to develop more efficient techniques, in particular for imaging native intracellular compartments in living cells. The results on TMA-DPH and DPH fluorescence microscopy will be combined with information obtained using label-free methods based on intrinsic fluorescence or absorbance of molecules upon excitation by DUV. Combining DUV fluorescence and absorption, two emerging techniques, will enable the acquisition of information on both organelle organization and chemical composition. Moreover, by using microfluidics devices, it will be possible to investigate in situ and in real time membrane and intracellular dynamics when cells are exposed to different kinds of environmental stress (osmotic, acid, oxidant, etc).





▸ *Figure 2: Saccharomyces cerevisiae cells stained with TMA-DPH (top) or DPH (low). TMA-DPH is mainly associated with plasma membrane and DPH with round intracellular structures corresponding to lipid droplets.*

▸ CONCLUSION

Biophysics of cell lipid structures is a historical research topic. Thanks to the high performance of synchrotron radiation combined with DISCO end-station, INRA and SOLEIL scientists are together developing innovative approaches based on DUV imaging. They gave access to dynamics and heterogeneity of membrane fluidity at the subcellular level. The current challenge is to improve experimental conditions and devices to achieve multimodal, label-free, microfluidics DUV imaging at SOLEIL.