



Challenges underlying the in situ analysis of single bacterial cells by synchrotron FTIR spectromicroscopy

Julie Meneghel, Stéphane Lefrançois, Stéphanie Passot, Frédéric Jamme,
Fernanda Fonseca, Paul Dumas

► To cite this version:

Julie Meneghel, Stéphane Lefrançois, Stéphanie Passot, Frédéric Jamme, Fernanda Fonseca, et al.. Challenges underlying the in situ analysis of single bacterial cells by synchrotron FTIR spectromicroscopy. European Conference on the Spectroscopy of Biological Molecules (ECSBM 16), Sep 2015, Bochum, Germany. hal-02269493

HAL Id: hal-02269493

<https://hal.science/hal-02269493>

Submitted on 5 Jun 2020

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Julie MENEHEL^{a,b,c}, Stéphane LEFRANCOIS^b, Stéphanie PASSOT^{b,a}, Frédéric JAMME^b, Paul DUMAS^b, Fernanda FONSECA^{a,b}

^a INRA, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78850 Thiverval-Grignon, France

^b AgroParisTech, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78850 Thiverval-Grignon, France

^c Synchrotron SOLEIL, L'orme des Merisiers, BP48, F-91191 Gif sur Yvette Cédex, France

julie.meneghel@grignon.inra.fr

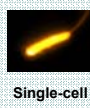


Introduction and context

Objectives

To achieve :

- An *in-line* biochemical monitoring of bacterial cells
- The characterization of single micron-sized bacteria
- Their analysis in physiological-relevant conditions.



Need for non-invasive & label-free techniques to analyze live single cells:
Raman & FTIR spectroscopies are good candidates

Spectroscopy	Raman	FTIR
Eukaryotic cells	Adherent cells. Subcellular resolution (organites)	Microfluidic devices: Tobin <i>et al.</i> , 2010, Vaccari <i>et al.</i> , 2012
Prokaryotic cells	Require the use of optical tweezers: Avetisyan <i>et al.</i> , 2013	Not achieved yet

Solution

➤ Synchrotron radiation SR-FTIR spectromicroscopy

Information provided on all relevant biological macromolecules:
membrane lipids, proteins, nucleic acids, carbohydrates



Low energy
MIR radiation
(4000-800 cm⁻¹ ⇔ 100-500 meV):
non destructive, does not harm biological samples

Bottlenecks

➤ Water absorption swamping the FTIR spectra if H₂O thickness > 10 μm (Tobin *et al.*, 2010)

➤ Spatial resolution: diffraction limited ± 3-8 μm, depending on λ
1 Bacteria ± 0.5-1 x 4-10 μm



Schematic size of *Lactobacillus delbrueckii* subsp. *bulgaricus* CFL1 cell (whey-based medium, 42 °C, late exponential growth phase)

Case study

Lactobacillus delbrueckii subsp. *bulgaricus*:
Lactic Acid Bacterium
Health benefits
Milk fermentation (yoghurt)



Fermentation

Concentration

Freezing

Storage

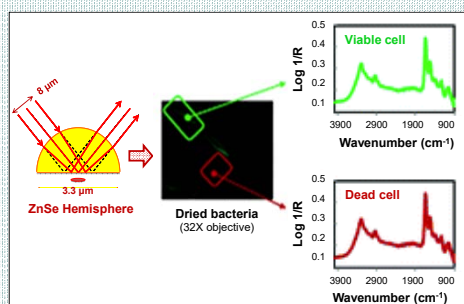
Final use

Viability & Functionality losses

First achievements

From dried samples...

SR-FTIR microspectroscopy in ATR-mode
+ Fluorescence staining

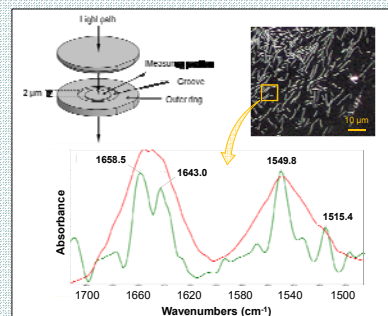


- ➔ Freeze-resistant cells ⇔ α-helices protein structures
- ➔ Freeze-sensitive cells ⇔ β-sheet protein structures

Passot *et al.*, 2015, *Analyst*

...to static liquid setup...

SR-FTIR microspectroscopy, transmission mode
+ CaF₂ liquid static cell



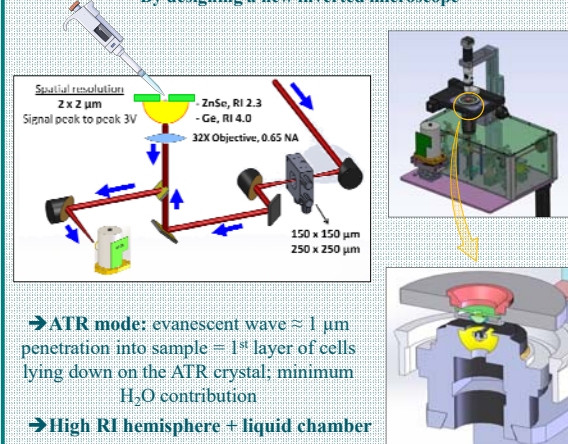
➔ First FTIR absorbance spectra of 5 - 6 bacteria in a liquid environment.

➔ Protein structures: fairly resolved

⚠ Measuring conditions are difficult to optimize

... towards liquid dynamic setup

By designing a new inverted microscope



➔ ATR mode: evanescent wave ≈ 1 μm penetration into sample = 1* layer of cells lying down on the ATR crystal; minimum H₂O contribution

➔ High RI hemisphere + liquid chamber

Perspectives

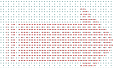
➔ Towards the first *in situ* analysis of single bacterial cells thanks to synchrotron FTIR spectromicroscopy

➔ **Ambition:** analysis of the stress-related damages in single *Lactobacillus delbrueckii* subsp. *bulgaricus* following production and end-using industrial processes



To increase our comprehension of the mechanisms involved: how does the set of a proteins, lipids, nucleic acids & carbohydrates of a bacteria react to an environmental stress such as osmotic, cold, oxidative, etc.?

To assess cellular heterogeneity within a bacterial population and to evaluate its relationship with stress-resistance?



Towards a better understanding of the underlying stress response mechanisms

