



**HAL**  
open science

## 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

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Julie MENEHEL<sup>a,b,c</sup>, Stéphane LEFRANCOIS<sup>b</sup>, Stéphanie PASSOT<sup>b,a</sup>, Frédéric JAMME<sup>b</sup>, Paul DUMAS<sup>b</sup>, Fernanda FONSECA<sup>a,b</sup>

<sup>a</sup>INRA, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78850 Thiverval-Grignon, France

<sup>b</sup>AgroParisTech, UMR 782 Génie et Microbiologie des Procédés Alimentaires, F-78850 Thiverval-Grignon, France

<sup>c</sup>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**.



Population  
10<sup>8</sup> - 10<sup>10</sup> cells/mL



Single-cell

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	<b>Not achieved yet</b>

### Solution

- > Synchrotron radiation SR-FTIR spectromicroscopy

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



High brilliance of the Synchrotron source for single-cell analysis

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

### Bottlenecks

- > Water absorption swamping the FTIR spectra if H<sub>2</sub>O 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

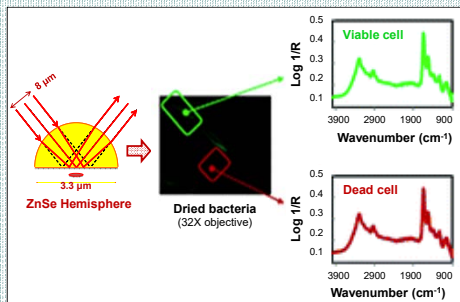
Final use

Viability & Functionality losses

## First achievements

### From dried samples...

SR-FTIR microspectroscopy in ATR-mode  
+ Fluorescence staining

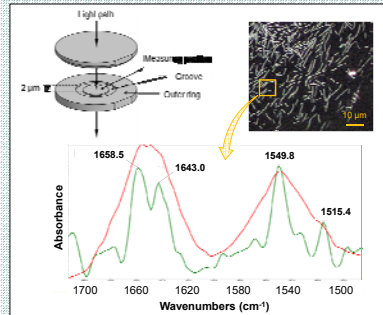


- **Freeze-resistant** cells ⇔ α-helices protein structures
- **Freeze-sensitive** cells ⇔ β-sheet protein structures

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

### ...to static liquid setup...

SR-FTIR microspectroscopy, transmission mode  
+ CaF<sub>2</sub> liquid static cell



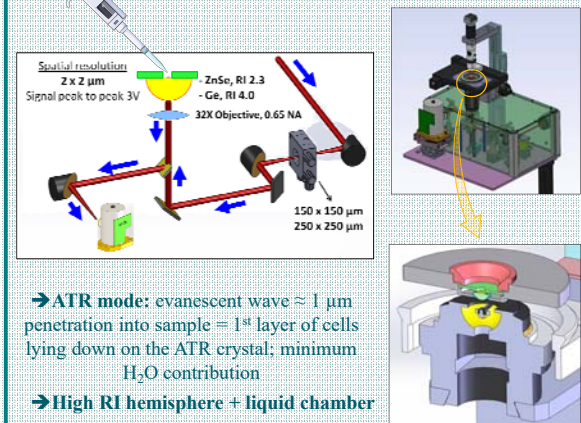
- **First FTIR absorbance spectra of 5 - 6 bacteria in a liquid environment.**

→ 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 H<sub>2</sub>O 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**

