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

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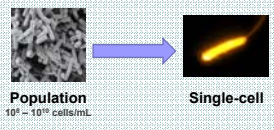
## Introduction and context

### 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 *et al.*, 2010, Vaccari *et al.*, 2012 |
| Prokaryotic cells | Require the use of optical tweezers: Avetisyan *et al.*, 2013 | **Not achieved yet** |

### Solution

- **Synchrotron radiation SR-FTIR spectromicroscopy**

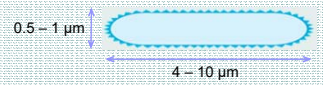
Information provided on all relevant biological macromolecules: **membrane lipids, proteins, nucleic acids, carbohydrates**

High brilliance of the Synchrotron source for **single-cell analysis**

Low energy MIR radiation (4000-800 $cm^{-1}$ ⇔100-500 meV): **non desctructive**, does not harm biological samples

### Bottlenecks

- **Water absorption swamping the FTIR** spectra if $H_2O$ 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)

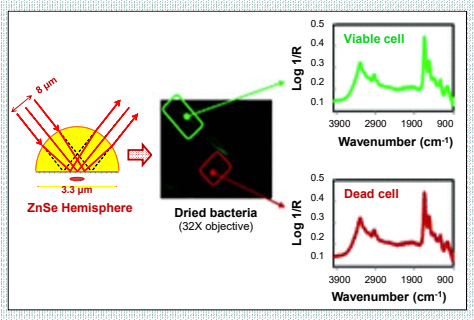
Fermentation → Concentration → Freezing → Storage → Final use

**Viability & Functionality losses**

## First achievements

### From dried samples…

**SR-FTIR microspectroscopy in ATR-mode + Fluorescence staining**



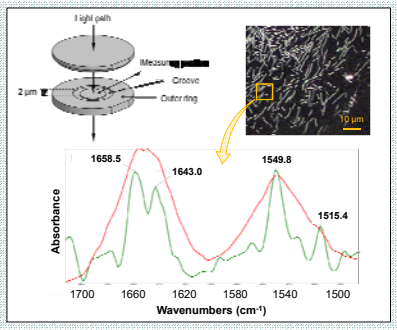

➔ **Freeze-resistant** cells ⇔ **α-helices** protein structures

➔ **Freeze-sensitive** cells ⇔ **β-sheet** protein structures

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

### …to static liquid setup…

**SR-FTIR microspectroscopy, transmission mode + $CaF_2$ liquid static cell**



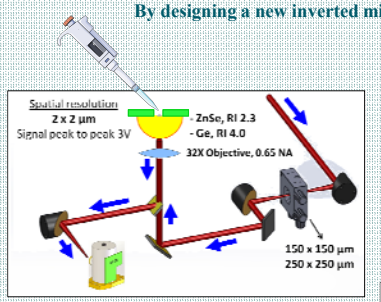

➔ **First FTIR absorbance spectra of 5 – 6 bacteria in a liquid environment.**

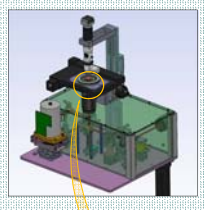
➔ Protein structures: fairly resolved

⚠ 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 = 1st layer of cells lying down on the ATR crystal; minimum $H_2O$ 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**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**Passot S, Gautier J, Jamme F, Cenard S, Dumas P & Fonseca F.** *Analyst*, **140** (2015), 5920-28.
**Tobin MJ, Puskar L, Barber RL, Harvey EC, Munro KL *et al.*** *Vib. Spectrosc.*, **53** (2010), 34–38.
**Vaccari L, Birarda G, Businaro L, Pacor S, Grenci G.** *Anal. Chem.*, **84** (2012), 4768–75.