Biochemical composition of micro-organisms at the single-cell level by synchrotron FTIR microspectroscopy

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SCIENTISTS INVOLVED

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KEYWORDS

Micro-organisms, biochemical composition, synchrotron-FTIR, single cell, microbial population heterogeneity, cell damage, metabolism.

Micro-organisms (yeast and bacteria) are exposed to different stressful environmental conditions. Sometimes, micro-organisms have to face undesirable stresses during their growth, when they are used in biotechnological processes (fermentation for production of biomass or molecules of interest), for stabilization and final use in food or green chemistry industries or when they are confronted to antimicrobial treatments used for eliminating undesirable micro-organisms. These different kinds of stress, including nutritional deficiency or excess, variations in temperature or in pH, osmotic stress, presence of antimicrobials, induce various physiological cell responses due to modifications in metabolism. However, the biochemical changes and the mechanisms of action involved are still not fully elucidated. Furthermore, the heterogeneity in cell response among the overall microbial population is largely unknown.

A better knowledge of individual cell behavior and the connection between cell biochemistry, biophysics and microbial physiology will make possible to produce homogenous cell populations through reverse engineering. According to the application and for ensuring maximal efficiency of the process under consideration, the homogeneity targeted will concern high lipid content, or high resistance to stabilization processes (freezing) or at the opposite, high sensitivity to antimicrobial treatments.
Fourier transform infrared (FTIR) spectroscopy is a non-invasive technique making possible to monitor biochemical changes in cells and tissues through their mid-IR vibrational modes. This powerful tool gives spectral fingerprints of biological macromolecules such as lipids, proteins, nucleic acids and carbohydrates,

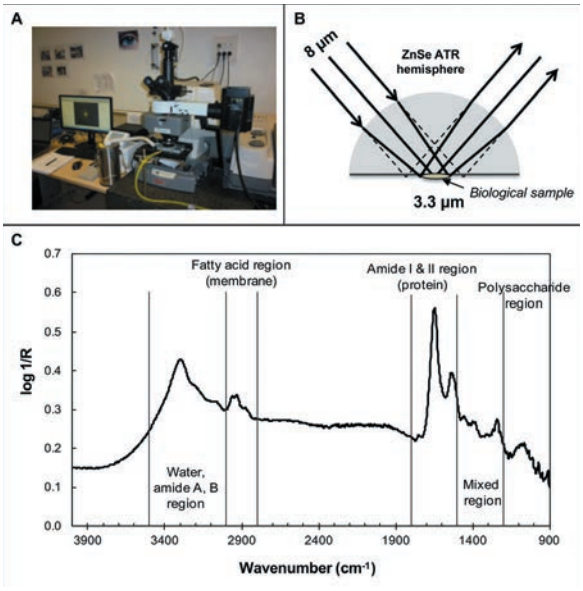
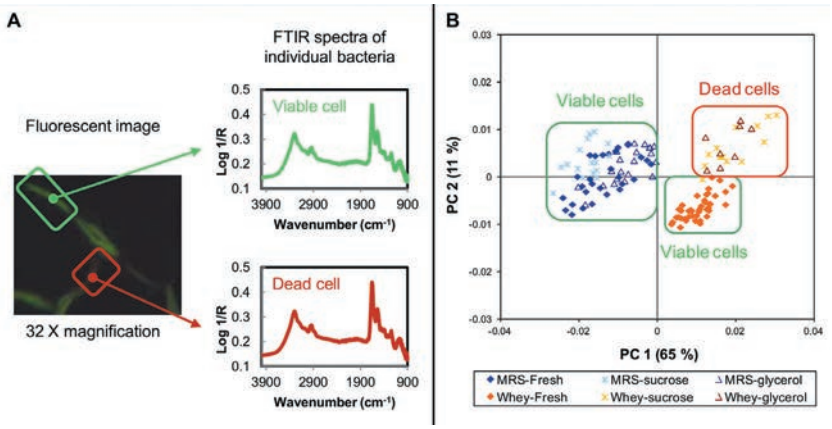


Figure 1: (A) Synchrotron FTIR micro-spectroscopy set up, (B) Schematic representation of the synchrotron light beam crossing the Zinc-Selenide (ZnSe) attenuated total reflectance (ATR) hemispherical element and (C) Representative synchrotron FTIR raw spectra in the 4,000-900 cm^{-1} region of individual microbial cells. Vibrations of characteristic molecular groups are indicated.

and is therefore sensitive to structural and compositional changes in cells. Due to the small size of micro-organisms (in the range of 1-5 µm), only the brilliance of the synchrotron-FTIR (SR-FTIR) beam allows the characterization of their biochemical composition at microbial-cell scale.

Pioneer work [1,2] assessed the changes in cell composition of the yeast *Saccharomyces cerevisiae* and the bacterium *Escherichia coli* after exposure to nano-silver coating or free ionic silver, respectively. By combining the use of SR-FTIR micro-spectroscopy (Fig. 1A) and high refractive zinc-selenide (ZnSe) hemispheres for a better spatial resolution (Fig. 1B), the authors achieved the unique challenge of analyzing biochemical composition at the single-cell level (3-5 µm) for microbial suspensions deposited and dried on the ZnSe hemisphere (Fig. 1C). In *S. cerevisiae*, a transition between active and inactive protein conformations was observed from the amide I peak downshift. Likewise, for *E. coli*, differences in cell composition were detected for proteins, but also for fatty acids as shown through the C–H stretching region. Furthermore, this approach enabled for the first time the assessment of heterogeneity in biochemical composition within cells among a given microbial population.

This innovative approach benefited to Froissard and collaborators, interested in studying the dynamics of lipid storage and cellular carbon fluxes in yeast to better understand metabolism heterogeneity and adaptation to stress induced by lipid over accumulation. Moreover, close collaboration with SMIS beamline scientists allowed the development of data pretreatment for reducing the resonance Mie Scattering, very disturbing for subsequent multivariate statistical analysis such as Principal Component Analysis (PCA) or Partial Least-Square Regression [3].



▶ *Figure 2: (A) Simultaneous assessment of physiological state and biochemical composition of single bacterial cell of L. bulgaricus. (B) Principal component analysis (PCA) of FTIR spectra obtained in the lipid region (3,000–2,800 cm^{-1}) after fermentation and after freeze-thawing of L. bulgaricus cells grown either in MRS broth (MRS, blue symbol) or in mild whey medium (whey, orange symbol) and protected by the addition of sucrose or glycerol.*

In order to deeper investigate the mechanisms underlying the cell responses to stress, complementary physiological approaches were developed at cellular level using specific fluorescent probes and analysis by flow cytometry [4]. Recently, an original work [5] reported the first bimodal analysis of bacteria at the single-cell level performed by combining SR-FTIR micro-spectroscopy and fluorescence microscopy at SMIS beamline (Fig. 2A). The simultaneous acquisition of information on the biochemical composition and the physiological state (enzymatic activity and membrane integrity) of *Lactobacillus delbrueckii* ssp. *bulgaricus* cells (*L. bulgaricus*), a bacterium widely used as a starter for manufacturing fermented and healthcare products, following fermentation and freezing allows to assess relevant spectral biomarkers of the cryotolerance of this bacterium. PCA analysis of SR-FTIR spectra indicated that before freezing, freeze-resistant *L. bulgaricus* cells grown in a rich medium (MRS) presented a high content of CH_3 groups from lipid chains, of cell proteins in an α-helix secondary structure and of charged polymers such as teichoic and lipoteichoic acids that constitute the Gram-positive bacterial wall. Physiological damages observed upon freezing with sucrose or glycerol as cryoprotectants, and leading to cell death, were ascribed to biochemical modification of cell membrane phospholipids, in particular to a rigidification of the cytoplasmic membrane following freezing (Fig. 2 B). Moreover, SR-FTIR micro-spectroscopy revealed cell heterogeneity within the cluster of freeze-resistant cells, which was ascribed to the diversity of potential substrates integrated from the growth medium.

▶ CONCLUSION

The multidisciplinary approach undoubtedly achieved major breakthroughs in the understanding of environmental stress impact on microbial populations. In particular, through investigations at the single cell level, the heterogeneity in cell response was highlighted, thus offering new avenues for more subtle physiological characterization, bioprocess optimization and design/combination of challenging tools at the interface between Biology and Physics.