Characterizing micro-organisms by two analysis methods: FT-IR spectroscopy and mass

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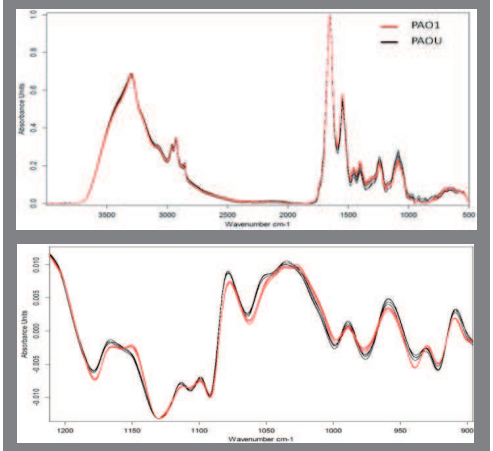
Introduction

Bacteria used to be characterized with mass spectrometry. However, this analysis method is limited when a wild type of Pseudomonas aeruginosa must be discriminated from its mutant. IR spectroscopy may have a higher power of discrimination than mass spectrometry. The module HTS_XT used,was an opportunity for the laboratory to realize bacterial spectra. In fact, this accessory was an important investment.

Experimental Conditions

FT-IR samples were prepared using some washes with NaCl (9g.L-1) on bacterial cultures which were adjusted at an optical density of 1. After these washes, the bacterial pellet was suspended in 70µL of sterile water. 5µL of this solution was deposited on the FT-IR plate and dried under vacuum for one hour. Then the plate was analyzed using a Tensor 27 (Bruker Daltonics).
MALDI-TOF spectra were acquired using a Microflex (Bruker Daltonics). Samples were prepared using the dry droplet technique and an HCCA matrix (α-cyano-4-hydroxycinnamic acid diluted in a mixture of H2O/CAN/TFA (50:50:2,5)). A bacterial colony was taken from agar plates and deposited on the plate to which 1µL of matrix was added. The analysis was calibrated on a reference spectrum of E.Coli DH5 α provided by Bruker.

Figure 1 : FT-IR spectra of two strains of Pseudomonas aeruginosa (PAO1 et PAOU) in absorbance (a) and in the first derivative (b)



Results and discussion

FT-IR spectra were obtained using a new protocol which was validated by analyzing nine strains of Pseudomonas aeruginosa and three strains of Lactoccocus lactis. Different strains were discriminated by transforming the spectra obtained to the first derivative spectra (cf.figure 1). Three strains of Pseudomonas aeruginosa were discriminated: PAO1, PAOU and Rhl a-. Two wild strains of Lactoccocus lactis were differentiated.
A database of Pseudomonas aeruginosa was created using the MALDI-TOF technique and the software BioTyper. Then, this database was tested by comparing other spectra. After this test, the reliability of the database for the strains : PAOU, Rhl I-, Rhl a- and H636 was demonstrated. One of these strains was discriminated from the others: PAOU. In fact, on its spectra, a peak was missing at 5400 Da. However, up to now, it's impossible to know to what it corresponds.
Strains of Lactoccocus lactis were also analyzed by MALDI-TOF spectrometry. At first, obtained spectra contained a few peaks but not enough to have a satisfactory bacterial fingerprint. So a bacterial spot treatment with TFA was tried. Different percentages were tested. To have more peaks in the spectra, 15% TFA was the most appropriate percentage. Even if the analysis was optimized it remained impossible to categorize the different Lactococcus lactis strains.
Nevertheless, the treatment of TFA opens a prospect to improve the database created for the Pseudomonas aeruginosa.

Conclusion

A new protocol for FT-IR analyses can be employed safely to realize bacterial spectra. Furthermore it was demonstrated that this method has a higher power of discrimination than MALDI-TOF spectrometry.

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