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Characterization of *Pseudomonas aeruginosa* biofilms grown on different substrates by means of FT-IR spectroscopy.

Marianna Portaccio , Alessandra Fusco, Giovanna Donnarumma and Maria Lepore
Department of Experimental Medicine, University of Campania *Luigi Vanvitelli*, Naples, Italy

AIM

In the present investigation, we aimed to characterize *Pseudomonas aeruginosa* biofilms grown on different substrates utilizing the Fourier Transform Infrared Spectroscopy (FT-IR), a non-destructive method allowing multiple analyses of the same biofilm largely adopted for this aim. *Pseudomonas aeruginosa* represents a class of bacteria largely investigated since it is an opportunistic pathogen, and it is now considered a primary infectious agent, especially for its ability to form multi-resistant biofilms. In particular, we investigated biofilms grown on Teflon membranes, CaF2 windows, and MirrIR slides (specific reflection FT-IR spectroscopy microscope slides)

MATERIALS AND METHODS

For the biofilm formation, the strain of *Pseudomonas aeruginosa* ATCC® 9027™ was grown overnight in Tryptone Soy Broth (TSB-Oxoid) at 37 ° C. The culture was then diluted at a concentration of 107 CFUs/ml, placed on sterilized CaF_2 disks, MirrIR slides and Teflon membranes and incubated overnight at 37 ° C in aerobic conditions.

For the IR characterization of the bacterial biofilm, different geometries were used for collecting spectra using the microscope stage of a Perkin Elmer Spectrum One spectrometer and a Universal Attenuated Total Reflection (UATR) device (Figure1). Microscope stage was used for the characterization of bacterial biofilms on CaF_2 disk (a transparent IR material) in transmission mode and biofilms adherent on MirrIR slides (IR reflective microscope slides) in transflection mode.

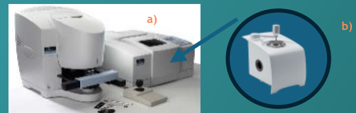
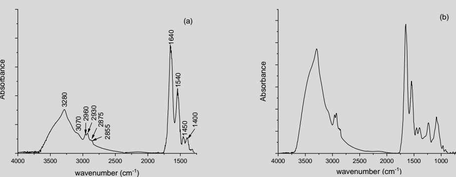


Figure 1: a) FT-IR Spectrum One with microscope b) UATR device.

The spectra of *Pseudomonas aeruginosa* biofilms on Teflon membranes were acquired in Attenuated Total Reflectance (ATR).

Multiple acquisitions of spectra were done, and statistical criteria were applied for monitoring and comparing them. To evaluate spectra reproducibility the correlation coefficients among the spectra acquired in analogous experimental conditions were estimated.



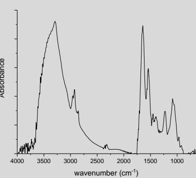


Figure 2. Average FT-IR spectrum of *P.* aeruginosa biofilm on: (a)Teflon membrane; (b) CaF2 support; (c) MirrIR support.

Table 1. Spectral position of main peaks of FT-IR spectra of Figures 2.

Peak position	Assignments	Description
3280	O-H and N-H stretching	polysaccharides and Amide A- proteins
3070	=C-H stretching	Unsaturated fatty acid
2960	C-H asym stretching	CH_3 of fatty acid chains and protein
2930	C-H asym stretching	CH_2 in fatty acid chains
2875	C-H sym stretching	CH_3 of fatty acid chains and protein
2855	C-H sym stretching	CH_2 in fatty acid chains
1640	C=O stretching C-N bending	Amide I - Protein
1540	N-H bending C-N stretching	Amide II-Protein
1450	C-H bending	CH_2 and CH_3
1400	C-O-H stretching	polysaccharides
1085	PO_2- sym stretching	nucleic acids and phospho-lipid
1050	C-OH stretching	polysaccharides
970	C-H bending	DNA backbone

RESULTS

The spectra of *P. aeruginosa biofilms* grown on different supports are reported in Figure 2. The most important peaks due to biological structures are evident and in Table 1 the positions of the main contributions are reported together with their assignments.

In the transflection mode using MirrIR supports, it is possible to obtain significative spectra in the 4000 to 650 cm^{-1} range allowing the study of contributions from all the components of bacteria.

For UATR acquisition geometry, the presence of Teflon peaks does not permit to obtaining valuable information for wavenumber smaller than 1300 cm^{-1}. Also, for transmittance collection geometry, it is possible to study the spectral region up to 1000 cm^{-1} due to the transmittance properties of CaF_2 in the infrared region. For these reasons, it is not possible to evaluate the contribution of DNA and polysaccharides, while the presence of lipids and proteins can be investigated without problems.

The bacterial biofilms obtained on the three different supports have different uniformity characteristics confirmed by optical microscopy images. In particular, the most uniform biofilms are obtained on CaF_2 windows and Teflon membranes, for which we also have the most reproducible spectra.

CONCLUSIONS

The above-reported considerations indicate that different choices are available in dependence on the measurement aims. For example, when the study of biofilm is focalized on bacterial proteins (important peaks in the range 1700-1500 cm^{-1}) the best choice in terms of speed of measurement and costs can be represented using UATR collection geometry with Teflon supports. Conversely, when the interest is focused on polysaccharides or nucleic acid bacterial components (range 1200-900 cm^{-1}) the use of the MirrIR support in transflection mode is suggested.

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