Multimodal fluorescence analysis of plant cell walls

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Introduction

Plant cell walls are abundant materials made of polymers that can potentially be turned into a wide range of chemicals, materials and fuels. But their chemical and structural complexity limits the efficiency of enzymes. In order to gain more knowledge in the architecture of plant cell walls, different experimental approaches are necessary. In particular, fluorescence spectroscopy and microscopy are relevant methods to decipher architecture, enzyme accessibility and interactions of plant cell wall polymers.

Materials and Methods

Poplar cell walls samples are submitted to different pretreatment (hydrothermal, dilute acid, ionic liquids) that will affect their structure in various ways. Some thin sections of 60 µm are prepared using a microtome in order to image the biomass fluorescence at several length scales: a spectrofluorimeter (Jasco FP-8300) for macroscopic mapping and a confocal microscope (Leica SP2) for acquiring cellular and tissular information.

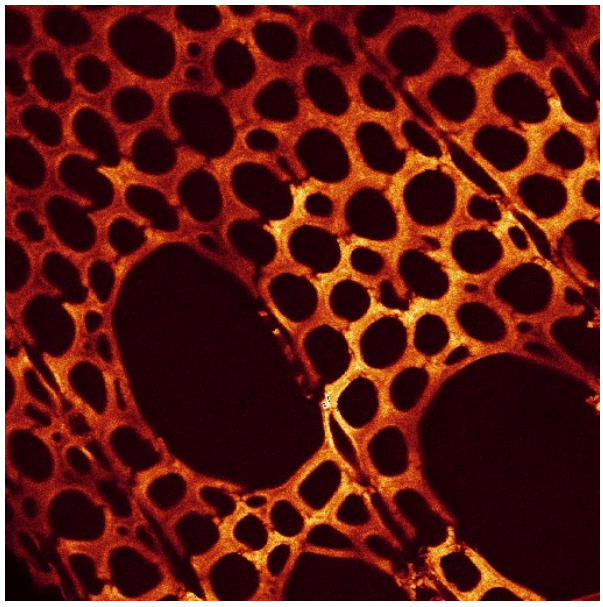

Figure 1. Native poplar section incubated for 24 hours in a 0.2% PEG-RhoB solution observed with a Leica SP2 confocal microscope

Results and Discussion

For each poplar cell wall sample, we have characterized:

- autofluorescence at wavelengths excitation/emission ranging on the ranges 200-600 nm/210-650 nm, respectively (3D fluorescence maps);
- autofluorescence mapping at cellular scale at selected wavelengths (351, 488 and 543 nm);

- fluorescence life-time microscopy (FLIM) mapping at cellular scale.

Fluorescence mapping is a straightforward and powerful tool to observe the impact of a pretreatment on poplar cell wall structure. The biomass fluorescence comes mostly from the lignin, which is one of the main fractions that has to be removed during the pretreatment step in order to increase cellulose accessibility.

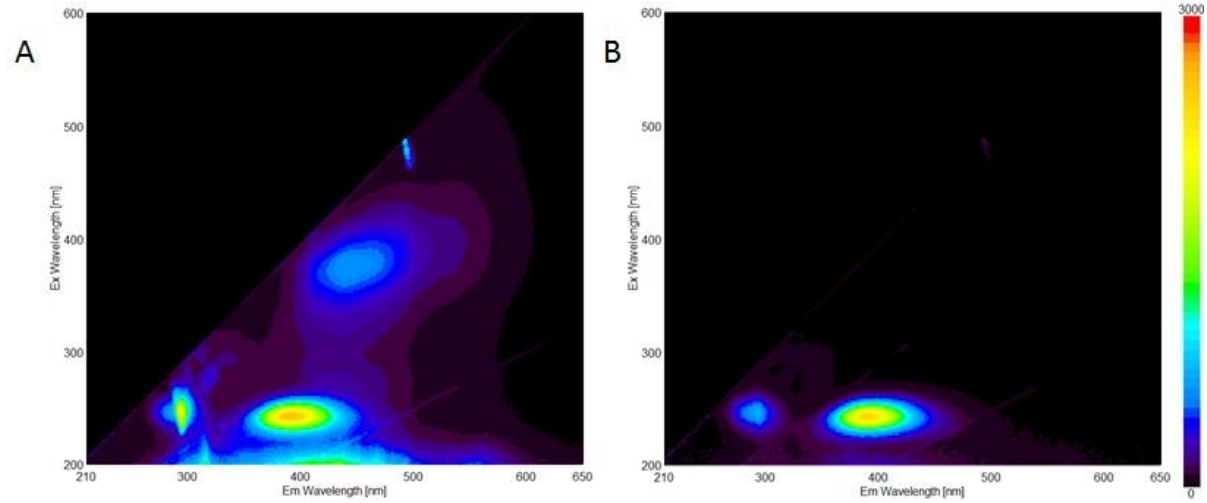

Figure 2. 3D fluorescence maps of native poplar (A) and dilute-acid-pretreated poplar (B).

As seen on figure 2, most of the fluorescence observed for native poplar is no more visible for dilute-acid-pretreated poplar. This shows that the pretreatment has an impact on the cell wall composition and structure.

This difference can also be observed by confocal microscopy. This technique can image the lignin fluorescence at the cell wall scale. By selecting the excitation and emission wavelengths, we can observe cell wall fluorescence, and the differences in the observed intensity give evidence of the pretreatment impact on lignin content (figure 3).

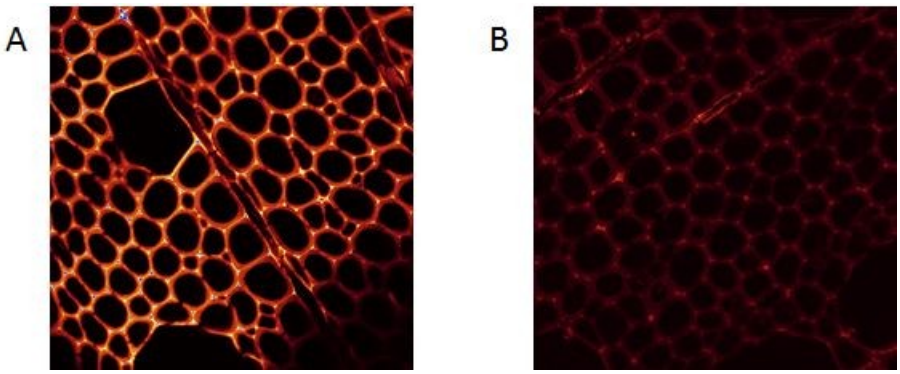

Figure 3. Confocal microscope images of native poplar (A) and hydrothermally pretreated poplar (B) sections.

Finally, FLIM information has been acquired on the same samples. FLIM is dependent on the environment of the fluorophores contained in lignin and thus highlight the interaction of such components. Average lifetime is increased by more than 25% in hydrothermally pretreated poplar sections compared to native poplar sections (Figure 4). This demonstrates that some modifications between linkages in lignin occurs during pretreatment.

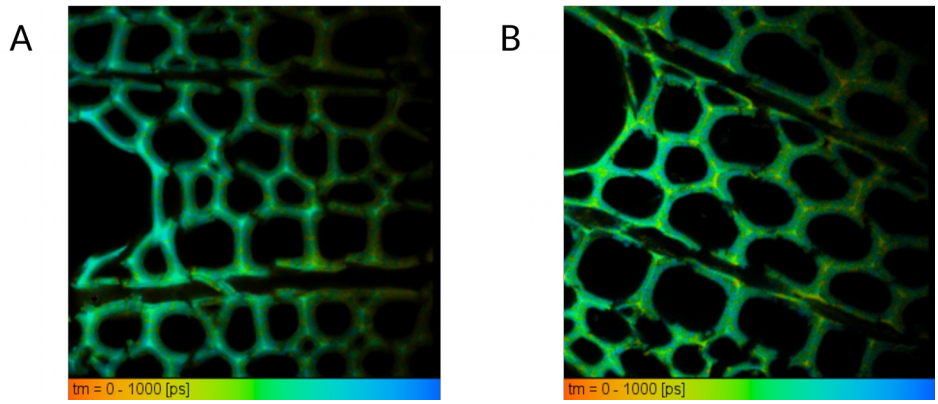

Figure 4. Confocal FLIM images of native poplar (A) and hydrothermally pretreated poplar (B) sections.

Conclusion

Fluorescence tools are versatile since they can reveal many features of plant cell walls (composition, architecture, interactions). Moreover, they require very small amounts of samples whose preparation is most of the time straightforward. In addition to the development of fluorescent probes to assess dynamical processes and interactions, they shape some state-of-the-art techniques to help solving the most challenging questions.

References

Paës G (2014) Fluorescent probes for exploring plant cell wall deconstruction: a review. *Molecules* , V.19, pp.9380-9402.