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CONTENTS

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CELLULOSE ORIENTATION AND PURITY ASSESSMENT AFTER TWO DIFFERENT PROCEDURES OF CELL WALL ISOLATION FROM MAIZE STEMS. A COMBINED MICROSCOPIC FLUORESCENCE DETECTED LINEAR DICHROISM AND IMAGE ANALYSIS STUDY

D. Djikanovic Golubovic[1], A. Savic[1], J. Simonovic[1], G. Steinbach[2], M. Jeremic[3], G. Garab[2], K. Radotic[1]

1. *Institute for Multidisciplinary Research, University of Belgrade, Kneza Viseslava 1, Belgrade, Serbia*
2. *Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, Hungary*
3. *Faculty of Physical Chemistry, University of Belgrade, Studentski Trg 12, 11000 Belgrade, Serbia*

Abstract

The effects on cellulose orientation and purity of two different methods for cell wall isolation from maize stems were investigated by using fluorescence-detected linear dichroism (FDLD) microscopy technique. Image analysis has shown that treatment with sulfuric acid have provided better results than previously applied method for cell wall isolation including enzymatic treatment.

Introduction

One of the most promising new sources for biofuel is "cellulosic ethanol", made from the cellulose contained in fibrous plant material such as cornstalks, grasses and forest trimmings. Maize is nowadays very important plant source for bioethanol. The main problem in obtaining pure cellulose is impossibility to separate it completely from lignin, due to very strong connection between these two polymers in the cell wall.

Linear dichroism (LD) of cell walls carries important information on their molecular organization. We measured LD of of the maize stem cell walls by differential polarization fluorescence imaging [1]. The samples were isolated by two different procedures. The cell wall fragments were stained by Congo Red which predominantly binds to cellulose, a major cell wall component that determines its anisotropy. By monitoring FDLD of the cell walls after various treatments, we could trace the degree of cellulose purity in the intermediate steps during cell wall isolation. FDLD image analysis show different degrees of anisotropy and purity of cellulose contained in the cell

wall. The structural changes were in parallel followed by using FTIR spectroscopy.

Material and methods

Cell walls were isolated from maize stem and purified according to a procedure of Chen *et al.* [2]. Other way of isolation of cell wall is with sulphuric acid [3] and then we treated sample with high pressure and strong mechanical force in FastPrep-24.

The samples were stained with Congo Red, which has been shown to intercalate into the cellulose matrix [4]. Samples were incubated for 2 h in 1 % (w/v) solution of Congo Red (Fluka) at room temperature.

Cell wall samples were measured in the differential polarization laser scanning microscope (DP-LSM) (Carl Zeiss Jena, Jena, Germany).

Results and discussion

During the imaging process, FDLD image and fluorescence emission image were stored as two separate channels. Sample was imaged by changing the focal planes with fixed increment. Sets of images were stacked in order to examine 3D structure of samples. As the basis for the surface rendering, fluorescence emission channel was used, while FDLD channel was used for object extraction based on threshold level settings.

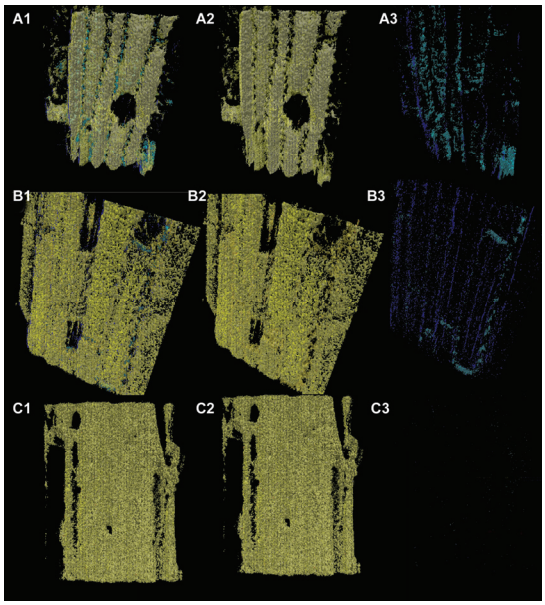

Figure 1. Rendered 3D images from the stacks of fluorescence emission images and FDLD images. Color scale: dark blue, 0^o polarization, gray, 45^o polarization, yellow 90^o polarization. 1, all objects presented, 2, extracted objects polarized from 45^o-90^o, 3, extracted objects polarized from 0^o-45^o A) untreated cell wall, B) plants treated with cellulaze pectinase, C) plants treated with sulphuric acid

The most important aim of the method was to examine if different structures have manifested the same or different FDLD values. The color bar scale of FDLD intensity was set from dark blue for the 0^o orientation, followed by gray for 45^o orientation, and finishing as yellow for 90^o orientation. For 3D image rendering software 3D Doctor was used.

Conclusion

Comparing the two cell wall isolation procedures, we obtained better results of cellulose purity for the maize stem treatment with acid. With this procedure horizontal elements in fiber structure were absent (Fig 1C3), which indicates that in

this case cellulose is more purified, i.e. more appropriate, for further procedure of ethanol production. In the process where the cell wall was treated with cellulase and pectinase, partially present horizontal structure remained.

These results are consistent with the changes in characteristic bands of cellulose and lignin in FTIR spectra of isolated cell walls in both procedures.

This procedure may be used for application in biofuels industry, in order to facilitate obtaining ethanol from cellulose fibers, which is the subject of our interest.

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