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Distribution of lignin and its coniferyl alcohol and coniferyl aldehyde groups in *Picea abies* and *Pinus sylvestris* as observed by Raman imaging

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ABSTRACT

Wood cell wall consists of several structural components, such as cellulose, hemicelluloses and lignin, whose concentrations vary throughout the cell wall. It is a composite where semicrystalline cellulose fibrils, acting as reinforcement, are bound together by amorphous hemicelluloses and lignin matrix. Understanding the distribution of these components and their functions within the cell wall can provide useful information on the biosynthesis of trees.

Raman imaging enables us to study chemistry of cell wall without altering the structure by staining the sample or fractionating it. Raman imaging has been used to analyze distributions of lignin and cellulose, as well as the functional groups of lignin in wood.

In our study, we observed the distribution of cellulose and lignin, as well as the amount of coniferyl alcohol and aldehyde groups compared to the total amount of lignin in pine (*Pinus sylvestris*) and spruce (*Picea abies*) wood samples. No significant differences could be seen in lignin and cellulose distribution between these samples, while clear distinction was observed in the distribution of coniferyl alcohols and coniferyl aldehyde in them. These results could provide valuable insight on how two similar wood species control biosynthesis of lignin differently during the differentiation of cell wall.

1. Introduction

Lignin in the wood cell wall has several functions. While being a binding component between individual cells, it also controls the water content inside the cell wall enabling transport of water and providing protection against pathogens as well as strength to load-bearing structures (Iiyama et al., 1994; Boerjan et al., 2003).

Lignin is commonly defined as a complex hydrophobic network of phenylpropanoid units derived from the oxidative polymerization of one or more of the three types of hydroxycinnamyl alcohol precursors (*p*-hydroxyphenyl, guaiacyl and syringyl units). Such definition, however, has been shown to be insufficient, for it is likely that no plant lignin derives solely from the three precursors (Sederoff et al., 1999).

Hypothetically, the structure of lignin is determined by the relative abundance of the precursors in the lignifying zone. This is controlled by genetic and environmental factors (Boerjan et al., 2003). One of the functional groups that has received relatively little attention from a structural perspective is coniferyl aldehyde (lignin-CAld), the reduction of which is considered to be the final step in the biosynthesis of coniferyl alcohols (lignin-CAlc) in lignin (Boerjan et al., 2003). Lignin-CAld groups prevail in native lignin since the activity of some coniferyl aldehyde dehydrogenase (CAD) enzymes is reduced during lignin biosynthesis (Boerjan et al., 2003). Coniferyl aldehydes have been shown to inhibit cell wall degradation by enzymes (Grabber, 2005), and they have been proposed to be a plant's response to a wound (Kim et al., 2003) or biotic and abiotic stress (Barakat et al., 2009).

To better understand the structure and function of lignin, a look at the broader composition of the wood cell wall is useful. Lignin is associated with other cell wall polymers, namely cellulose, hemicellulose and pectin. The latter two polymers combine with lignin to form the individual cell binding matrix known as the middle lamella whereas cellulose is the most important load-bearing component of the cell wall. Cellulose in plants is found as semi-crystalline aggregates, microfibrils, whose orientation along the fiber axis varies depending on their location in the cell wall.

During the cell formation, the biosynthesis forms lamellar cell wall layers which differ from each other in contents of structural components and their alignment. The division of a cell wall into two major layers is fairly established: the secondary cell wall resides next to the hollow lumen, which is further divided into three layers S3, S2 and S1, and outside the primary cell wall layer forms a thin cover for the cell. The structure of wood cell wall is schematically depicted in Fig. 1 which clearly shows the division in different cell wall layers and changes in cellulose microfibril orientation.

The distribution of lignin and other structural components of the wood cell wall has commonly been studied by using optical

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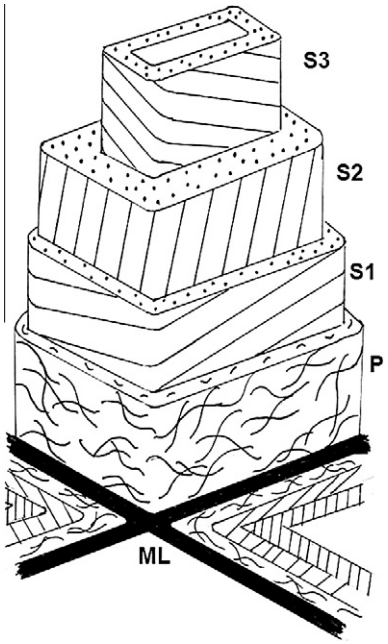

Fig. 1. Schematic representation of the structure of wood cells. Individual cells are separated by middle lamella. The cell wall is divided into two layers, primary wall (P) next to middle lamella (ML) and secondary wall which is further divided in S1, S2 and S3 layers. A hollow lumen is surrounded by the cell wall layers.

(Ritter, 1925; Wardrop, 1965; Scurfield, 1967; Côté et al., 1968; Peng and Westermark, 1997) and electron microscopy (Fernando and Daniel, 2008; Terashima et al., 2009) or preparative fractionation of cell wall sublayers (Marton and Adler, 1961; Whiting and Goring, 1983; Sorvari et al., 1986; Sundberg et al., 2002). When a wood sample is fractionated or modified to be suitable for microscopic analysis, mechanical or chemical treatment is applied to the sample. This can cause changes in the cell structure and modify cell wall components introducing artifacts to the sample. By using spectroscopic methods, such as infrared (IR) and Raman spectroscopies, measurements can be performed on the samples without any preparatory steps that could alter them. Measurements on wood in its native state are possible with Raman spectroscopy, because the Raman response of water is very weak due to its low polarizability, i.e., water does not dominate over the Raman spectrum and therefore disturb the measurement. In contrast, an IR spectrum is significantly influenced by the presence of water in the sample since IR absorption is pronounced for polar molecules.

Limits of resolution in IR become more undesirable when considering spectroscopic imaging. In spectroscopic imaging, for example Raman imaging, spectra are collected at regular, spatial intervals from the selected area on the sample. From the collected spectra, images are constructed according to the heights or areas of the selected bands. Such images illustrate changes in chemical structures or distributions of different components in the sample. Raman imaging has been shown to provide informative results when interpreting the cell wall ultrastructure of wood (Agarwal, 2006; Gierlinger and Schwanninger, 2006; Schmidt et al., 2009; Gierlinger et al., 2010) and other plant (Gierlinger and Schwanninger, 2007) samples.

Earlier very few studies on the distribution of lignin-CAld (and lignin-CAlc) groups in wood cell walls (Wardrop and Davies, 1959; Peng and Westermark, 1997; Pomar et al., 2002) have been conducted. In the present study, we used Raman microscopy to determine the distribution of lignin and, more importantly, the distribution of lignin-CAld and lignin-CAlc groups in the cell wall of two softwood species, Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*). Clear differences could be distinguished in cell wall level, which has not been shown earlier. These two species are the most common and industrially most important softwood species in the Nordic countries. The direct visualization of various components and functional groups within the cell wall is bound to yield fresh knowledge on their role in plants. Furthermore, by understanding the variations in chemical distributions of cell walls, industrial processes can be tailored more specifically for certain species. For example, lignin-CAld contents in wood have been shown to affect the result of chemical pulping (MacKay et al. 1999).

2. Results and discussion

2.1. Lignin/cellulose distribution

A Raman spectrum consists of bands which are caused by an inelastic scattering from the chemically bonded structures. Cellulose, hemicelluloses, pectins and lignin all contribute to the Raman spectrum collected from wood. Raman spectra from the secondary cell wall of spruce and pine are presented in Fig. 2A and B to illustrate the complexity of wood spectrum. Cellulose and lignin provide the most prominent Raman bands, while hemicelluloses and pectins have remained undetected due to their low content, broad Raman bands and overlapping with stronger bands of other components (Agarwal and Ralph, 1997). Spectra of pure model components have been measured in several studies in order to detect their contributions in the Raman spectra of lignocellulosic substances (Agarwal and Ralph, 1997, 2008; Wiley and Atalla, 1987; Saariaho et al., 2003a,b; Nuopponen et al., 2004a,b).

Polarized Raman spectra of polymers are sensitive to orientation (Koenig, 1999), which must be considered in the interpretation of their spectra. Especially the Raman spectrum of cellulose has been shown to change in different orientations (Atalla et al., 1980), while the spectrum of lignin shows only small changes (Atalla and Agarwal, 1985). The sensitivity toward orientation in Raman bands of cellulose has also been applied to determine the microfibril angle in different cell wall layers, as shown in a recent study by Gierlinger et al. (2010). To avoid misinterpretation of the results due to orientation, our measurements have been conducted so that the measured area contains cell walls that are along and perpendicular to the polarization of the excitation laser, in the images vertical and horizontal cell walls, respectively.

When analyzing images consisting of the intensity of certain bands only, it is necessary to understand that the presented val-

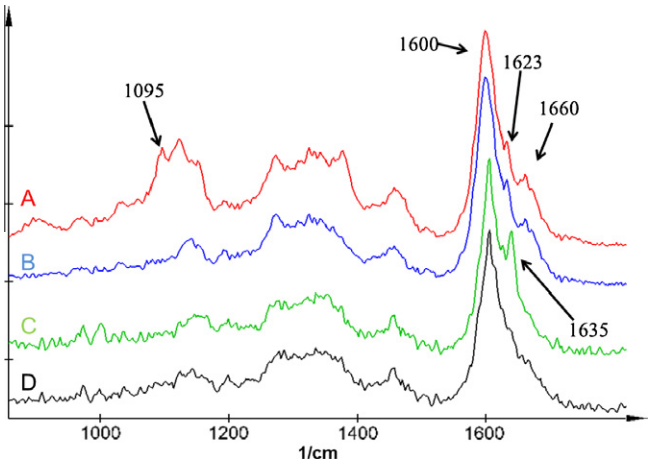

Fig. 2. Raman spectra from S2 cell wall (A) and cell corner middle lamella (B) of pine. Spectra from middle lamella of $NaBH_4$ treated pine (C) and spruce (D) samples. Spectra have not been normalized. Spectra shown in figure are averages of several spectra collected from data used for images. Spectra of single measurements are presented in Supplementary material Fig. S2 online.