Micron 43 (2012) 916–919

Contents lists available at SciVerse ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Short communication

Correlative light and scanning electron microscopy of the same sections gives new insights into the effects of pectin lyase on bordered pit membranes in *Pinus radiata* wood

Mark West*, Alankar Vaidya, Adya P. Singh

Scion, 49 Sala Street, Private Bag 3020, Rotorua 3046, New Zealand

ARTICLE INFO

Article history:
Received 15 November 2011
Received in revised form 7 March 2012
Accepted 7 March 2012

Keywords:
Bordered pit
Pectin lyase
Toluidine blue
Correlative microscopy
Pinus radiata

ABSTRACT

Bordered pits are structures in the cell walls of softwood tracheids which permit the movement of water between adjacent cells. These structures contain a central pit membrane composed of an outer porous ring (margo) and an inner dense and pectin-rich disc (torus). The membrane is overarched on each side by pit borders. Pits may be aspirated, a condition where the torus seals against the pit border, effectively blocking the pathway between cells. In living trees this maintains overall continuity of water conduction in xylem by sealing off tracheids containing air. Drying of timber results in further pit aspiration, which reduces wood permeability to liquid treatment agents such as antifungal chemicals. One possible way to increase permeability is by treating wood with pectin lyase to modify or remove the torus. The effectiveness of this treatment was initially evaluated using light microscopy (LM) of toluidine blue stained wood. Pectic material is coloured pink-magenta with this stain, and loss of this colour after treatment has been interpreted as indicating destruction of the torus. However, correlative light (LM) and scanning electron (SEM) microscopic observations of identical areas of toluidine blue stained sections revealed that many unstained pits had intact but modified tori when viewed with SEM. These observations indicate that LM alone is not sufficient to evaluate the effects of pectin lyase on pit membranes in wood. Combining LM and SEM gives more complete information

1. Introduction

Softwood trees transport water and minerals tangentially and axially through tracheids in the outer sapwood (Tyree and Zimmermann, 2002). This transport process involves structures called bordered pits, which occur in the wall between adjoining tracheids. In softwood trees such as radiata pine (*Pinus radiata*), bordered pits contain a central pit membrane composed of an outer porous ring (margo), and an inner dense and pectin-rich disc (torus) (Imamura et al., 1974; Daniel et al., 1996). The membrane is overarched on each side by pit borders. In radiata pine trees, water can readily flow across the pit via the membrane, particularly through the more highly porous margo. However, when air is introduced into a tracheid, pit membranes become closed (aspirated) when the torus lodges against the pit border. This prevents the air from spreading into adjacent tracheids, thereby maintaining the continuity of the column of water from the base to the top of the tree. Aspiration of pit membranes also occurs during the formation of heartwood or when the timber is dried (Harris, 1991; Singh et al., 2009). This greatly restricts the flow of liquids and adversely affects impregnation of the timber with treatment solutions such as chemicals used to protect against decay. Pit membrane destruction by physical, chemical, biological and enzymatic treatments can greatly improve wood permeability. The effects of these different treatments also provide information about the structure and chemical composition of pit membranes (Bauch et al., 1970; Tschernitz, 1973; Meyer, 1974; DeGroot and Sachs, 1976; Militz, 1993; Liese et al., 1995; Daniel et al., 1996; Kobayashi et al., 1998; Schwarze and Landmesser, 2000; Hong-Hai et al., 2005; Schwarze et al., 2006; Singh et al., 2009).

To enhance permeability of radiata pine wood, we are evaluating an environmentally friendly process of treating the wood with enzymes to destroy the pit membrane torus, which consists largely of pectic substances (Daniel et al., 1996). The aim is to achieve a permeable wood substrate that permits easier ingress of substances used to enhance wood properties and appearance. To this end, we have monitored the effect of enzymes on radiata pine pit membranes using correlative light and scanning electron microscopic observations of the same microtome-cut sections.

* Corresponding author. Tel.: +64 7 343 5406; fax: +64 7 343 5444.
E-mail addresses: mark.west@scionresearch.com (M. West), Alankar.vaidya@scionresearch.com (A. Vaidya), adya.singh@scionresearch.com (A.P. Singh).

0968-4328/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2012.03.007

Correlative light and electron microscopy approaches give complementary information and have been used on a wide range of biological samples (Gaietta et al., 2006; Sartori et al., 2007; Schwarze and Humbel, 2007), including observations on identical sites of the same sections to obtain information at different resolutions (Saglie et al., 1985; Kushida et al., 1993). A correlative microscopy approach has recently been used for wood and wood products, observing the same sections by light, confocal laser scanning, and scanning electron microscopy (Singh and Dawson, 2006), or by field emission scanning electron microscopy operating in secondary electron and backscattered electron imaging modes (Singh et al., 2007). These observations revealed the intricate distribution of a protective coating in the surface layers of saw-textured coated radiata pine plywood panels. Singh et al. (2010) used a combination of light microscopy and field emission scanning electron microscopy in secondary electron and backscattered electron imaging modes to visualise chitosan impregnation of radiata pine wood cells and differentiate between cell wall and lumen penetration.

In this communication we describe a technique for correlative light and scanning electron microscopy of bordered pit membranes using the same sections of enzyme treated radiata pine wood. Toluidine blue stains pectin-rich structures such as pit membrane tori pink-magenta (O'Brien and McCully, 1969). Light microscopy employing this stain can be used to examine the tori of bordered pit membranes in wood, and their presence or absence in response to different treatments. However, reliance on light microscopy alone may lead to erroneous conclusions. By combining light and scanning electron microscopy to examine the same areas in a section we have gained new insights into the effects of enzymes on bordered pit membranes.

2. Materials and methods

The wood used in this study was fresh sapwood from *Pinus radiata* D.Don. Sections were prepared by splitting off the radial longitudinal surface of a narrow face (about 3 mm) of wood with a razor blade and then taking 60 μm sections at a slight angle to the fractured surface with a sledge microtome. This yielded sections with part of the surface cut and part fractured. The complete pit membrane is frequently exposed on fractured (but not cut) surfaces because the overarching pit border is removed, thus allowing a clearer view of its structure. Sections were kept in water prior to enzyme treatment.

Sections were incubated for varying lengths of time in a commercial pectin lyase preparation (Novozyme Bioprep® 3000L) at 2% (v/v) in 100 mM sodium phosphate buffer, pH 8.0, at room temperature. A control sample was incubated in buffer for the longest time period of treatment. At the end of the treatment time, sections were washed thoroughly in water, stained in a 0.1% aqueous solution of toluidine blue for 1 min and rinsed before mounting in water on a glass microscope slide.

Sections were viewed with an Olympus BX61 microscope equipped with an Olympus ColourView III digital camera. Immediately after light microscopy the sections were carefully removed from the glass slides, briefly dipped in 100% ethanol, clamped between two glass slides and dried under vacuum overnight.

For scanning electron microscopy, samples were mounted on aluminium stubs using adhesive carbon tabs, and coated with chromium (Emitech K575X sputter coater). Microscopy was carried out using a JEOL 6700F FESEM at an accelerating voltage of 3 kV. Light microscopy images were used to locate the pits to be viewed with SEM.

Alignment of LM and SEM images was done using Adobe Photoshop CS5 software.

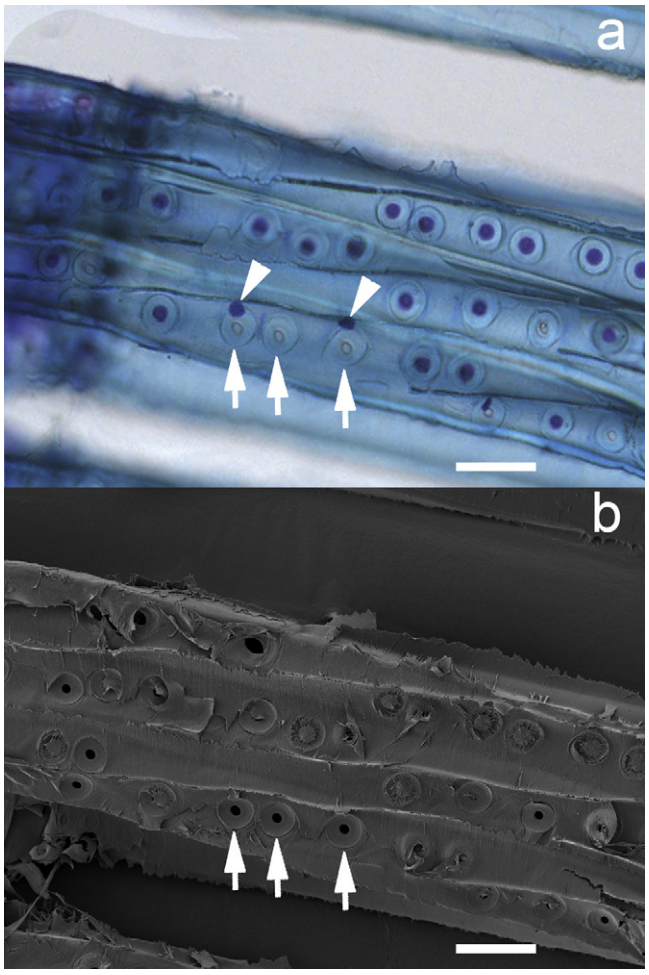

Fig. 1. (a) Light microscope image of toluidine blue stained untreated wood section. Arrows indicate pits without staining in the torus region. Arrowheads indicate stained displaced tori. (b) FE-SEM image of the section shown in 1a. Arrows indicate absence of tori from pits that did not stain with toluidine blue. Bar = 50 μm.

3. Results and discussion

3.1. LM and SEM observations of untreated (buffer control) wood

Images of a representative toluidine blue-stained untreated wood section observed by LM and SEM are shown in Fig. 1a and b, respectively. The pink-magenta colour of the majority of the tori in Fig. 1b indicates their intactness. However, the absence of stained tori in some pits (Fig. 1b, arrows), indicates the loss of the pit membrane. Pit membranes are fragile structures, and even in untreated (control) samples some may be lost due to tearing and fracturing during sample preparation, as the presence of displaced tori in Fig. 1a (arrowheads) would suggest. The corresponding SEM micrograph (Fig. 1b) confirms the presence of tori in those pits where a pit membrane was detected by light microscopy. These observations demonstrate the usefulness of LM combined with toluidine blue staining for visualising pit membrane tori and determining their presence or absence in bordered pit structures.

3.2. LM and SEM observations of pectin lyase treated wood

LM and SEM images of the same area of a representative section from a wood block treated with pectin lyase for 30 min are shown in Fig. 2a and b, respectively. In Fig. 2a the central areas (the inner circles) of pit membranes corresponding to tori are not stained with toluidine blue, suggesting complete loss of tori. However,