Action of *Sporotrichum pulverulentum* on Wood Cell Walls

KATIA RUEL and JEAN-PAUL JOSELEAU

Centre de Recherches sur les Macromolécules Végétales,
CNRS-Grenoble, B.P. 68, 38402 St. Martin d'Hères cedex, France
Tel. 76-54-11-45

Abstract

A study by electron microscopy of the mode of degradation of wood cell walls by *Sporotrichum pulverulentum* wild type and mutants revealed several aspects of the wall digestion. Specific labelling of the different polysaccharide constituents with enzyme-gold markers, localization of the sites of attack by controlled silver staining were employed in an attempt to characterize the biochemical mechanisms involved in the fungal attack. Two distinct mechanisms seem to be prevalent whether the hyphae are in direct contact with the cell wall, or whether there is a distance between the hyphae and the site of the degradation. This could correspond to two phases in the degradative action of the fungus.

1. Introduction

Sporotrichum pulverulentum is amongst the most efficient wood-rotting fungi. As a white-rot species it bears the capacity of degrading lignin as well as cellulose and the other cell wall polysaccharides (Eriksson, 1981) in a simultaneous attack. The microorganism degrades cellulose by the synergistic action of several endo- and exo-1,4-β-glucanases in connection with an oxidation enzyme, cellobiose oxidase, and with the cellobiose-quinone oxido-reductase (Eriksson and Hamp, 1978). The latter enzyme has been

shown to be involved in both cellulose and lignin degradation (Westermark and Eriksson, 1974). The micromorphological action of *S. pulverulentum* on wood cell walls has been followed by electron microscopy (Ruel and Barnoud, 1985). This technique together with the use of cellulase-less mutants of the fungus was used in the present study in order to acquire details about the progressive removal of lignin and hemicelluloses.

The investigation was carried on spruce wood (*Picea abies*) the cell walls of which contain the three main types of hemicelluloses, xylan, glucomannan and galactoglucomannan in close association with lignin and cellulose.

2.   Materials and Methods

Organisms

1.   *Sporotrichum pulverulentum* Novabranova P 1271 (ATCC 32629), referred to as wild type (Synonymous: *Phanerochaete chrysosporium*) (Burdsall, 1981).

2.   Cellulase repressed *Sporotrichum pulverulentum* obtained by cultivation on glucose impregnated wood samples (Eriksson and Hamp, 1978).

   (a)   Cultivation of the fungi on wood. Blocks ($20 \times 20 \times 10$ mm) of Spruce (*Picea abies*) sapwood were cultivated as described in Ruel et al., (1981).

   (b)   Preparation of samples for electron microscopy. Matchstick like samples ($1 \times 1 \times 5$ mm) were cut from the decayed spruce wood and embedded in methacrylate or glycol methacrylate (GMA) (Ruel and Joseleau, 1984) after fixation with $KMnO_4$ or glutaraldehyde. In order to localize the polysaccharide moiety of the wall, three techniques were used:

   (i)   The periodic acid-thiocarbohydrozide-silver proteinate (PATAg) method as modified by Ruel et al. (1977).

   (ii)   Mannanase or xylanase-gold complexes (Ruel and Joseleau, 1984) prepared with Au_5 gold particles obtained by reducing chloroauric acid with white phosphorus.

   (iii)   ETAg method (Joseleau and Ruel, 1985) using purified xylanase (EC 3.2.1.8.) or mannanase (EC 3.2.1.78) for creating reducing groups along xylan (or mannan) chains which were visualized with silver proteinate.
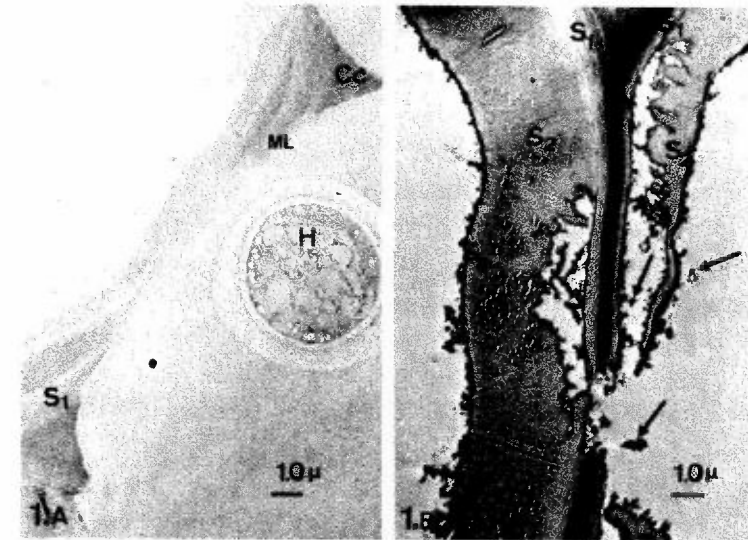
Figure 1. Two patterns of degradation by the wild-type strain A : Au_5 Mannanase colloidal-gold complex; B : $KMnO_4$ fixed sample. A. Hypha in close contact with the remaining middle lamella (ML) and cell-corners (CC). The entire wall has been digested. No residue is visible. B. No hypha visible but the wall is strongly degraded particularly the S_2 layer. $KMnO_4$ positive electron dense granules are left (arrow).

3.   Results and Discussion

From the examination of a great number of photomicrographs from several samples of spruce wood decayed by the wild type strain of *S. pulverulentum*, we came to the conclusion that the fungus does not exhibit a unique pattern of degradation. Two extreme aspects are shown in Fig. 1. In photograph A, the action of the hyphae in close contact with the cell wall results in a complete digestion of all the wall polymers. The highly lignified middle lamella seems to be the most resistant part of the wall although it will eventually

be dissolved, leading to isolated cell corners. It has to be noted that in this type of degradation the wall constituents are immediately metabolized by the hyphae, the cell corners being the last to disappear. A completely different pattern is shown in photograph B where, whereas no hyphae can be seen in the vicinity, the degradation proceeds gradually in the S_2 layer of the wall. In this case shreds of the internal part of S_2 remain. In other patterns different kinds of micromorphological aspects could be observed, wedge openings with accumulation of electron dense residual granules, or swelling, leaving a fibrillar material (Ruel et al., 1981).

The capacity of the fungus to achieve such high levels of degradation of the different polymer constituents of the wall is due to the great diversity of its enzyme equipment. A demonstration of the first removal of hemicelluloses before lignin is provided in Fig. 2 in which sections were stained respectively with PATAg reagent for polysaccharides (A) and potassium permanganate for lignin (B). The unstained halo visible around the hole is due to the removal of hemicelluloses (A). On the other hand the remaining material of the halo is constituted of a highly reactive lignin as indicated by the strongly positive staining with $KMnO_4$ (B).

The characterization of the mode of action of the fungus on two chemically distinct polymers, lignin and polysaccharide, is easier than to visualize the specific removal of a given polysaccharide. However the situation can be helped by the comparison of the mode of action of modified strains lacking cellulase activity but having strong ligninolytic activity. In this case the cellulose network remains, but, as shown by the shadowing image (Fig. 3A) undergoes a dissociation of the microfibrils which results in a strong swelling. The specific removal of lignin and of the hemicelluloses in S_2 is illustrated by the low glucomannan content left in the degraded walls, specifically labelled (Fig. 3B) by a gold-mannanase complex (Ruel and Joseleau, 1984). The progressive removal of the hemicelluloses further evidenced by the newly developed technique ETAg (Joseleau and Ruel, 1985) suggests that the concomittant solubilization of lignin and hemicelluloses leads to marked loosening of the cellulose microfibrils interconnection. The weak density of staining of both xylans and glucomannans, Fig. 4A and 4B respectively, along the cellulose microfibrils is due to the interconnecting position of the hemicelluloses between the microfibrils which makes their total removal difficult.

In conclusion the diversity of the patterns of action of *Sporotrichum pulverulentum* must be related to the degree of accessibility of the wall components to the enzymes released by the hyphae. In a close contact all the
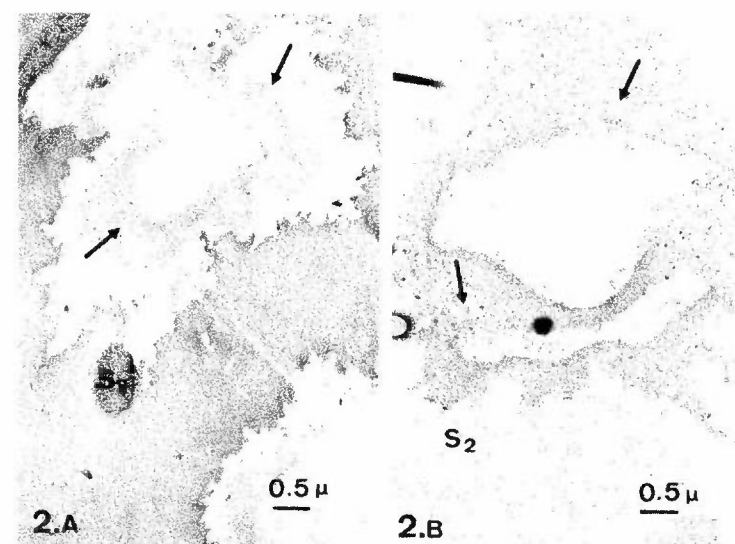
Figure 2. Sequential solubilization of polysaccharides and lignin by the wild type strain. A — $KMnO_4$ fixation. A. a PATAg negative halo is surrounding the hole (arrow). The remaining wall exhibits a PATAg positive staining. B. The unstained material in Fig. A is strongly $KMnO_4$ reactive (arrow) which clearly indicates that lignin is still present.
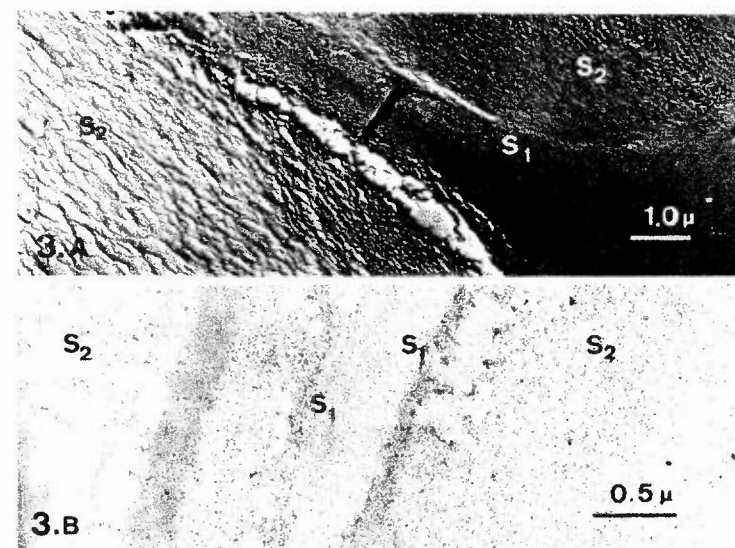

Figure 3. Cell walls degraded by the cellulase-repressed strain. A — Shadowing. B — Au_5 — Mannanase-gold complex. A. The solubilization of the lignin and glucomannan part of the wall reveals the trellis-like organization of the cellulose network. B. The glucomannan content is particularly low on the fibrillar structures. However the outer part of S_2 seems less easily degraded.
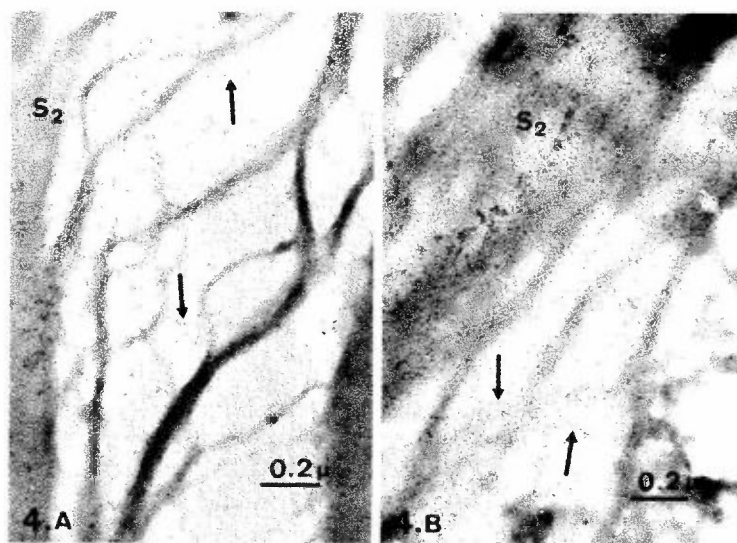
Figure 4. Characterization of the remaining polysaccharides after degradation by the cellulase-repressed strain. A. ETAg-xylanase. B. ETAg-Mannanase. Even after a strong solubilization of lignin and hemicelluloses, xylan and glucommanan are still found on the cellulose microfibrils in their ultimate morphological state.

material can be easily degraded and metabolized *in situ*. At a distance from the hyphae, because of the difficulty of penetration of the enzymes in the compact composite cell wall where hemicellulose, cellulose and lignin are very closely entangled, the images of degradation correspond to only a partial removal of the wall polymers.

The various patterns could also be interpreted as the consequence of variations in the nature of the induced enzymes in the hyphae.

Acknowledgements

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