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Degradation of pruning wastes by *Phanerochaete sordida* growing in SSF: Ultrastructural, chemical, and enzymatic studies

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ABSTRACT

A solid standard fermentation (SSF) with the fungus *Phanerochaete sordida* in a medium with *Nephrolepis cordifolia* (entire pinnae separated from the rachis) and *Laurus nobilis* (fragmented leaves) was performed over 92 days to study the degradation of leaves with histological, chemical, and enzymatic methods. The fungus entered the leaves early, through the stomata in *N. cordifolia* and *L. nobilis*, and also through mechanical cuts that had been made in the latter. The initial attack affected the mesophyll in both plant species, and the phloem in *L. nobilis*. The vascular bundle of *N. cordifolia* was protected by a sheath of cells with thick lignified walls. The collenchyma cell walls situated near the principal vein in *L. nobilis* swelled during the initial stages of enzymatic action, but reduced their thickness afterwards, mainly in regions of contact with the hyphae. At the end of the experiment, no species had leaves with mesophyll. In *L. nobilis*, phloem was also lacking, and a partial and heterogeneous attack on the xylem became evident. The histological changes are compared with the enzymatic activities and the chemical composition of the culture media, describing the stages of fungal colonization.

1. Introduction

Problems caused by municipal solid waste (MSW) have become more severe in recent years as a result of increasing amounts of waste and decreasing availability of landfill space (Alter, 1991; Chefetz et al., 1996). For the organic fraction, biodegradation seems to be the desirable treatment; it has the advantage not only of reducing the volume and weight of the wastes but also of providing composted organic residues for soil structure, thereby increasing soil fertility and plant growth (Giusquiani et al., 1995).

The composition of MSW can vary with climate, standard of living, type of garbage collection system, and season. The city of Buenos Aires, for example, currently produces about 4000 tons day^{-1}, and of this amount, nearly 96.4 tons day^{-1} are organic residues, mainly consisting of grass clippings, leaves, and, to a lesser extent, wood chips. In broad terms, the organic residues represent 11% of the total production of MSW from a city, with seasonal fluctuations of 6–20% (Johansson et al., 1986). The management of organic residue degradation would seem to call for low technology (Haug, 1995), but the slow rate of decomposition and the large space that is required deter potential investors.

Residues with similar characteristics are found in litter in nature. Detritus transformation is a process of key importance in the cycling of elements in ecosystems. In forests, litter is generally the primary source of nutrients for plants and microorganisms. The filamentous fungi play an important role in litter degradation, since many species can degrade the biopolymers present in plant tissues with extracellular enzymes, such as pectinases, xylanases, cellulases and ligninases, that facilitate substrate penetration (Eriksson, 1988; Asiegbu et al., 1996). The mature regions of the fungal hyphal wall are rigid, providing considerable mechanical strength and allowing a high level of turgor pressure, so that high hydrostatic pressure can be exerted at the hyphal tip. Such pressure assists the hypha in forcing its way through plant tissues. This rigidity, together with the hydrolytic action of the exoenzymes, favours the typical invasive growth of the filamentous fungi (Gooday, 1995).

In the search of methods that would optimise the efficient degradation of green residues, different techniques of solid-state fermentation (SSF) have been applied. In compost, for example, the natural succession of the microorganisms involved in the degradation of the organic matter can be accelerated by the control of nutritional and physical parameters (Giusquiani et al., 1995). As a result, there is an important loss of the lignocellulosic components (Nusbaumer et al., 1996). Fungal monoculture or mixed cultures have also been used to favour a rapid degradation (Blanchette and Shaw, 1978a,b; Braga et al., 2002). The main factors in the control of composting include physical environmental parameters (temperature, moisture content, pH, aeration) and natural substrate

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parameters (C/N ratio, particle size, and nutrient content) (Díaz et al., 2002). The airflow resistance of compost is closely related to the porosity, free airspace, and bulk density of the compost pile. The air content is just as important for providing an oxygen supply, as it is to remove carbon dioxide and excessive moisture and to limit heat accumulation (Haug, 1995). Particle size determines the porosity of the pile and the surface available to microbes for degradation; by decreasing the particle size, a greater surface area is exposed to microbial attack. Although many studies have examined these factors, only a few have investigated the structure of the residues, and even fewer have focused on the leaf fractions. This lack of information may be due to the fragility of this plant organ under fungal attack and the difficulty of studying the degradation processes.

This paper deals with leaf decay of *Nephrolepis cordifolia* and *Laurus nobilis* by the fungus *Phanerochaete sordida* grown under SSF conditions, focusing on changes in leaf ultrastructure and chemical composition caused by fungal penetration and enzymatic (pectinolytic, amylolytic, cellulolytic, and ligninolytic) activities.

2. Materials and methods

2.1. Microorganism

P. sordida (BAFC 2122) was maintained at 4 °C on malt extract agar (malt extract, 1.3%; glucose, 1%; agar, 2%) slants and subcultured every 4 months.

2.2. SSF conditions

The culture medium included mature entire pinnae, separated from the rachis, of *N. cordifolia* (50% dry weight) and fragments of mature leaves of *L. nobilis* of 2–3 cm (50% dry weight). Leaves were collected during the winter of 2005 from home gardens in Buenos Aires, Argentina. The water content was adjusted to 60% with a solution (25 g l^{-1}) of $SO_4(NH_4)_2$. Culture media (70 g) were placed in plastic cylindrical jars (10 cm wide and 15 cm high), and autoclaved (20 min, 121 °C). Twenty-six flasks were inoculated with a 5-mm^3 cube taken from the edges of the colony of *P. sordida* grown for 5 days on malt agar at 28 °C without light. The flasks were maintained in a dark culture chamber at 28 °C. Three uninoculated flasks were exposed to the same environmental conditions as controls. Samples were extracted at various stages of the decomposition process.

2.3. Histological methods

2.3.1. Light microscopy

The material was fixed in formaldehyde:alcohol:acetic acid (FAA), dehydrated, embedded in paraffin wax, sectioned, stained, and mounted following standard histological techniques (D'Ambrogio, 1986).

2.3.2. Transmission electron microscopy (TEM)

The material was pre-fixed in 3% glutaraldehyde in phosphate buffer (pH 7.2) at 2 °C for 24 h, and then post-fixed in OsO_4 (1.5%) in the same buffer at 2 °C for 3 h. Samples were then dehydrated in an ethanol series and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate (O'Brien and McCully, 1981) and observed and photographed with a JEOL-JEM-1200 EX II microscope.

2.4. Analytical methods

2.4.1. Measurement of pH

Ground dried solid material was suspended in distilled water (1/10) for 20 min with sporadic shaking. The pH was measured with a glass electrode attached to the pH meter.

2.4.2. Reducing sugars, proteins and enzyme determination

Extracts were made with 700 mg of fresh samples suspended in 10 ml of sodium acetate buffer 0.1 M (pH 4.8), shaken for 20 min, and centrifuged at 2000 rpm for 15 min. The reducing sugars were assayed following Somogyi (1952) and Nelson (1944). The soluble proteins were assayed by the method of Bradford (1976) using BSA as the standard. Polygalacturonase (3.2.1.15, poly 1,4-α-D-galacturonide) was assayed by using 0.1% polygalacturonic acid in a 50 mM acetate buffer (pH 4.8) as a substrate at 50 °C. For endoxylanase (3.2.1.8, β-1,4-D-xylan-xylanohydrolase) determination, samples were incubated at 50 °C in a 0.1 M sodium acetate buffer (pH 4.8) containing xylane (0.2%) as substrate. Endoglucanase (3.2.1.4, endo-1,4-β-glucanase) was assayed at 50 °C in 0.1 M sodium acetate buffer pH 4.8 using 0.5% CMC as a substrate. The total amylase activity was measured at 50 °C in a 0.1 M sodium acetate buffer pH 4.8 using 2% soluble starch as substrate. The products of hydrolytic enzymes were determined according to Somogyi (1952) and Nelson (1944). Laccase activity was determined in 0.1 M sodium acetate buffer (pH 3.5) by using 5 mM ABTS as a substrate. The reaction product was monitored at 420 nm ($\varepsilon_{420} = 36\ mM^{-1}\ cm^{-1}$) (Bourbonnais et al., 1995). One enzyme unit was defined as 1.0 μmol of product formed per min. Enzyme activity was expressed as U g^{-1}dry weight of culture media. For water extract component determination, samples of 20 g dry weight were milled with a mortar and washed in a Soxhlet extractor using distilled water as a solvent; 14 washing cycles were applied. The solid washed material was placed in a stove at 80 °C for 48 h and weighed. The holocellulose and lignin were determined by hydrolysis with H_2SO_4 following the method proposed by Effland (1997). The remnant fraction after the hydrolysis represented the lignin content. All compounds were purchased from Sigma.

Determinations were made five times on samples at time zero (not inoculated with the fungus) and on cultures 92 days after inoculation. The data obtained were expressed as a percentage of the dry weight loss ± standard deviation.

3. Results

3.1. Ultrastructural changes in leaves during degradation

After a brief phase of adaptation that lasted approximately 30 h, the fungus began to grow vigorously, covering the leaf surfaces of *N. cordifolia* and *L. nobilis*. This rate was maintained until day 15 of its growth, when all the cultured leaves had been invaded superficially.

Following day 2, the first hyphae entering inside leaf structure were observed. *P. sordida* hyphae penetrated through *N. cordifolia* stomata (Fig. 1a), and also through the mechanical cuts made in tissue of *L. nobilis*. By day 20, 100% of the foliar remains of the culture medium exhibited extensive colonization.

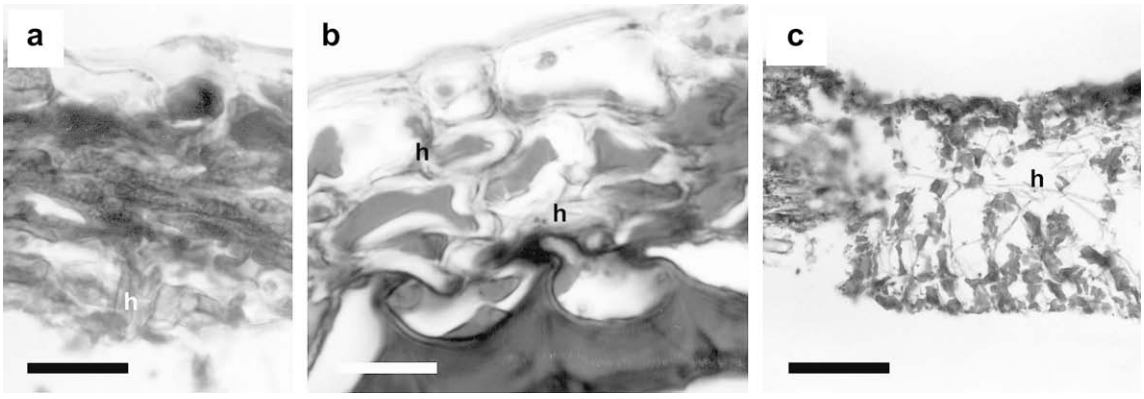

Fig. 1. Light microscopy photographs of transverse sections of *N. cordifolia* and *L. nobilis* leaves: (a) hypha entering through a stoma in *N. cordifolia*; (b) hypha growing through the middle lamella of parenchyma cells in *N. cordifolia*; (c) hypha invading the mesophyll in *L. nobilis*. Bars = (a) 20 μm; (b) 16 μm; (c) 150 μm.

The first manifestation of the attack within leaves was detected in the mesophyll of both plant species. This attack was more rapid in *L. nobilis* due to the larger entering surface offered by the mechanical cuts of the fragments (Fig. 1c). The hyphae progressed inside the leaves' intercellular spaces and then invaded the middle lamella, which caused the separation of the mesophyll cells (Figs. 1b and 2a). Primary walls were degraded next at interfaces with the hyphae (Fig. 3a and b). This last event facilitated the invasion of the hyphae into the interior of the cells where the reduction of their cell wall content began (Fig. 3c). Concomitantly with the attack to the mesophyll, the deterioration of the radial and internal tangential epidermal walls was observed, leaving only the cuticle in many cases (Fig. 2a). In *L. nobilis* the walls of the collenchyma cells that surrounded the vascular bundle swelled as an indicator of the initial enzymatic action, and reduced their thickness afterwards, mainly at interfaces with the hyphae. After 60 days, the mesophylls of both plants were totally degraded. The attack to the phloem of *L. nobilis* began by day 5, and that tissue was completely destroyed by day 18 (Fig. 2a). In *N. cordifolia*, where a sheath with thick and lignified secondary walls surrounds the vascular bundle (Fig. 1b), the phloem was not attacked.

Although hyphal growth was observed in the lumen of the xylem vessels of *L. nobilis* after day 16, the first signs of degeneration appeared at day 20. The hyphae progressed in the xylem across the cell lumina; the dissolution of the walls began with thinning of cell walls and reduced electron-density after observations with TEM (Fig. 2a). Cell wall penetration was observed (Fig. 3b) and eventually penetration of the hyphae inside the cells (Fig. 3c). Different patterns typical of fungal attack were distinguished in the xylem. "T" figures were observed, characteristic of middle lamella degradation (Fig. 2b). Safranin staining was weaker, which provided evidence of lower lignin concentrations (Fig. 2b) and the secondary wall thinning of vessels (Fig. 2b).

By day 92, the leaves of both species were deprived of whole mesophyll cells and almost the entire epidermis with only the epidermal external tangential walls and the cuticle remaining. In addition, *L. nobilis* exhibited a lack of phloem, a reduction in the collenchyma surrounding the vascular bundles, and a partial heterogeneous attack to the xylem. In contrast, the vascular tissue was intact in *N. cordifolia*.

3.2. Exoenzymes activities of fungi growing on leaves

After infection with *P. sordida*, soluble proteins increased in SSF to reach a first peak at day 22 and a second one of lower intensity at day 64 (Fig. 4). Residual sugars showed three peaks – on days 15, 43, and 78. Sugar peaks corresponded to peaks of the tested polysaccharide degrading enzymes (Figs. 4 and 5a).

The polygalacturonase activity was maximum between days 22 and 50 (Fig. 5a), which coincided with mesophyll and phloem attack. The amylase showed three peaks of activity, the major one at day 50 and two smaller ones on days 22 and 78 (Fig. 5a). The endoxylanase activity, responsible for the cleavage of the plant cell walls hemicelluloses, increased rapidly, reaching a peak on day 29, at which time the phloem and the mesophyll were already invaded (Fig. 5a and b). At day 43, after xylem invasion had started, the endoxylanase activity had decreased by 50%. The endoglucanase activity responsible for the rupture of cellulose peaked on days 29 and 50. The laccase activity slowly rose to reach a maximum on day 29, which corresponds to the initial phase of invasion of xylem, a tissue rich in lignin (Fig. 5c).

The pH of the culture medium decreased from an initial value of 6.7 ± 0.36 to 5.5 ± 0.28 during the first 15 days, and then maintained an average of 5.6 ± 0.42 until the end of the experiment.

3.3. Chemical changes in leaves

The dry weight loss, the hydrosoluble components, and the holocellulose and lignin content were determined on the final samples of the culture medium. The culture medium lost $68.2 \pm 4.4\%$ of total dry weight. The hydrosoluble component was reduced by $79.6 \pm 6.9\%$. The reduction of holocellulose, mainly comprised of hemicellulose and cellulose, averaged $70.5 \pm 4.2\%$. The final lignin content was reduced to $8.1 \pm 0.83\%$ with respect to the initial values. No variation of the initial values was observed after 92 days for the uninoculated control cultures (without *P. sordida*).

4. Discussion

The fungus *P. sordida* is capable of invading leaves of *L. nobilis* and *N. cordifolia* and degrading a great part of their tissue when cultivated in SSF conditions. The *L. nobilis* leaves contain terpenoids and essential oils that confer resistance to the attack of microorganisms (Cowan, 1999), but evidence exists that many fungi which may overcome those barriers (Hintikka, 1996). The results presented here suggest that this is the case for *P. sordida* on the tested substrates.

Owing to its chemical characteristics, the cuticle represents a natural barrier for the fungus, compelling the fungus to start the attack by way of the stomata or in ruptured zones; this phenomenon was also observed in *Sporotrichum pulverulentum* growing on leaves of *Fagus sylvatica* (Rihani et al., 2001). This confirms the need to use previously cut or mechanically damaged leaves as substrates in SSF to facilitate hyphae penetration.

Based on histological examination of degraded tissues and evolution of enzyme activity, the degradation process can be

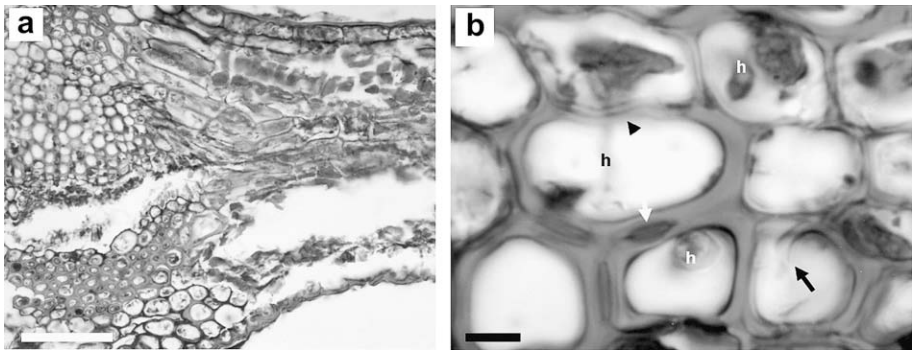

Fig. 2. Light microscopy photographs of transverse sections of leaves of *L. nobilis*: (a) parenchyma and phloem attacked; (b) detail of the xylem. Middle lamella (white arrow), thinned wall with weak reaction to stains (black arrowhead), broken walls (black arrow) and hyphae (h) invading the cellular lumen. Bars = (a) 100 μm; (b) 10 μm.

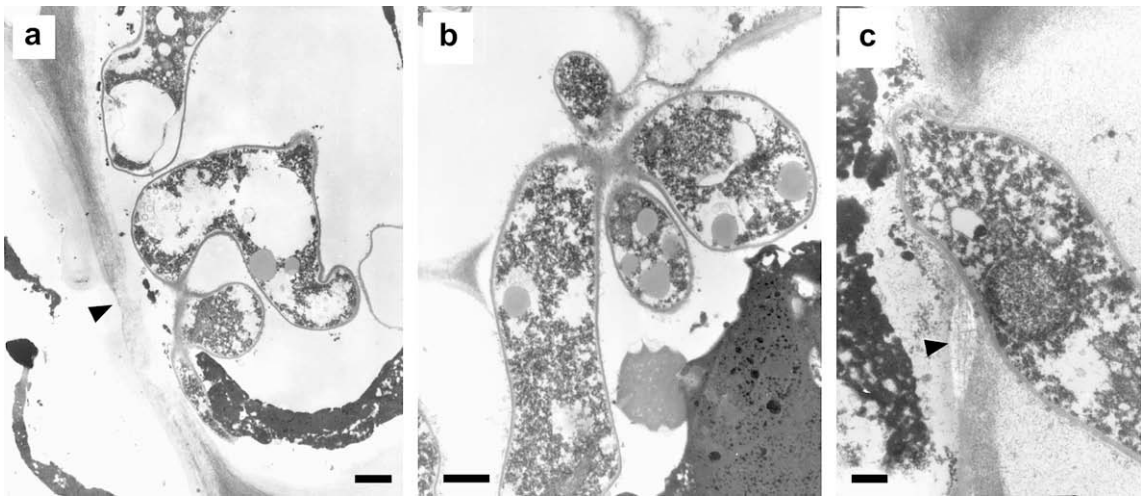

Fig. 3. Transverse section of the xylem of *L. nobilis* photographed with TEM: (a) hyphae growing in contact with the thinned wall (arrowhead); (b) hyphae in contact with the almost completely degraded wall; (c) thinned middle lamella and hypha crossing the perforated wall. Bars = (a) 1 μm; (b) 1 μm; (c) 0.5 μm.

divided into two stages. The first stage, which corresponds to days 0 to about 25, is characterized by an invasion of the entire substrate (both the surface and the interior), while the enzyme activities involved in degradation increase. During this first stage, degradation occurs mainly in the more exposed (and, consequently, more easily accessible) polysaccharides that comprise the middle lamella, and the invasion encompasses most of the tissues composed of thin primary cell walls rich in cellulose content, such as the parenchyma and phloem. Then in a second stage of the biodegradation process, the tissues containing lignified secondary walls are degraded. During this phase, initially (between days 30 and 50), the xylanolytic and cellulolytic activities drop, ceding ground to the lignolytic enzymes, whose function is to uncover the less exposed polysaccharides. Then, between days 55 and 65, the xylanolytic and cellulolytic activities increase to degrade the newly exposed polysaccharides. During these phases the hyphae enter and grow throughout the natural spaces of the material, which are the main paths of colonization.

The degradation stages are related to the progression of nutrient availability. *Phanerochaete chrysosporium* and *Trametes versicolor* growing on wheat straw first attacked the non-lignified tissues, such as the parenchyma and phloem, independently of the pattern of degradation that followed (Barrasa et al., 1995). The same was observed in *P. chrysosporium* and *Ganoderma zonatum* during wood degradation of a palm tree (Adaskaveg et al., 1991). It seems to be a common phenomenon, also reported for other fungi, such as *Ceriporiopsis subvermispora* and *Cyathus stercoreus* during the

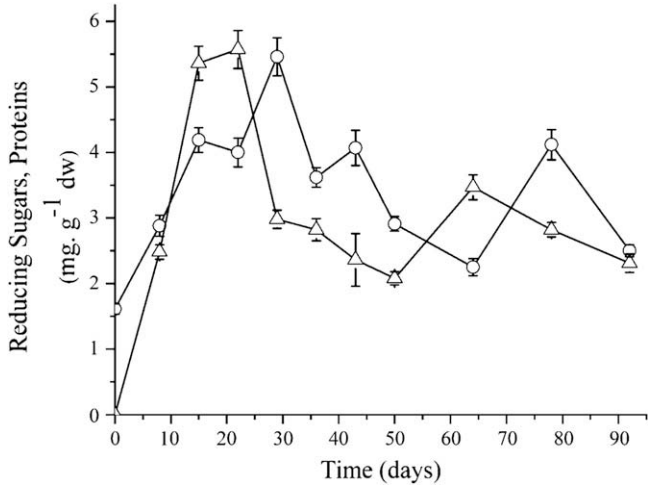

Fig. 4. Reducing sugars and soluble proteins determined in *P. sordida* during its growth in SSF. Open circles, reducing sugars; open triangles, soluble proteins. Values shown are the means ± S.E.M. for five determinations.

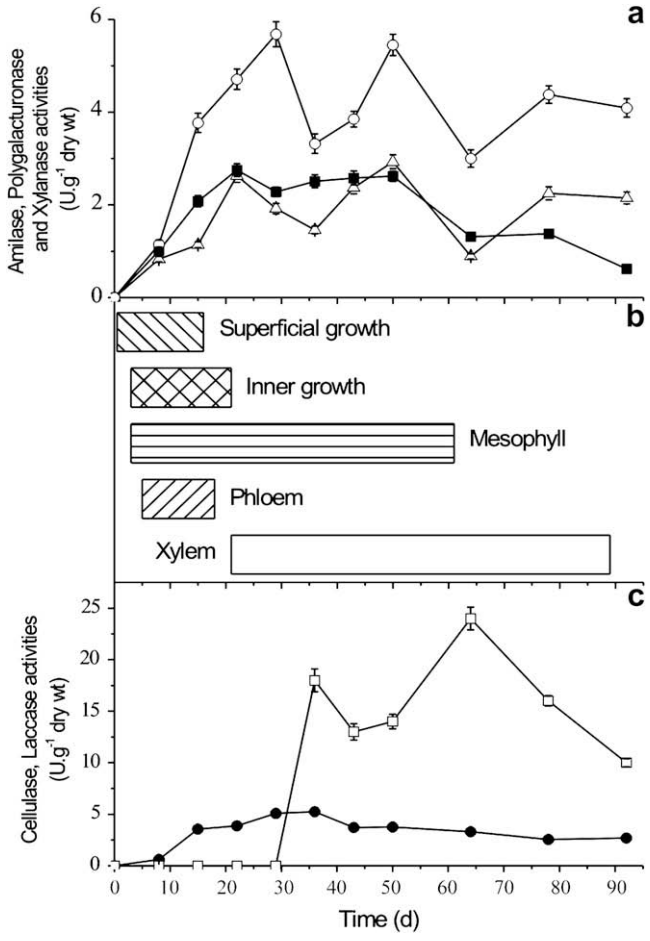

Fig. 5. Relation between the enzymatic activities and growth of *P. sordida* as a function of time: (a) activities of the enzymes amylase, polygalacturonase, and endoxylanase; (b) periods of surface growth, inner growth, and degradation of different plant tissues; (c) activities of the enzymes cellulase and laccase. Open triangles, amylase; closed squares, polygalacturonase; open circles, xylanase; closed circles, laccase; open squares, cellulase. Values shown are the means ± standard deviation for five determinations.

decomposition of lignocellulose belonging to herbaceous plants (Akin et al., 1995). Rihani et al. (2001) reported a more complex mode of degradation of *F. sylvatica* by *S. pulverulentum*, in which hemicellulose and lignin of parenchyma cells and fibres were degraded concomitantly during early stages of invasion, whereas primary and secondary walls and middle lamella were attacked at more advanced stages of decay.

In the case of *L. nobilis* and *N. cordifolia*, sclerenchyma degradation by *P. sordida* is slower and incomplete. The attack of lignified tissues (wood) may be selective or simultaneous (Blanchette et al., 1985; Davis et al., 1994). In the former, the lignin is attacked but the cellulosic wall structure is preserved. In the latter, the nonselective attack produces an erosion of the wall without extensive lignin loss throughout the cells. This difference in mode of attack, depending on the wall structure, reflects the diversity in the proportion of lignin and its chemical nature (Migné et al., 1996). Grabber et al. (1997) showed that the quantity of *p*-hydroxyphenyl, guaiacyl, and syringyl in lignin directly affected the degradability of the cell wall by hydrolytic enzymes. Ruel et al. (1994) reported that strains of *P. chrysosporium* showed different patterns of attack whether they grew on hardwood or softwood, suggesting that the structural components of the substrate influences the pattern of degradation. The incapacity of entering the sheath of lignified walls of *N. cordifolia* and the vessels of *L. nobilis* would indicate differences due to lignin type, which might be related to the degrading capacity of the fungus.

This work increases the scant bibliographic sources about degradation of foliar remains. The combination of methods from experimental mycology and plant anatomy permits correlation of the decay of the leaf tissues with the enzymatic activities and chemical composition of the substrate, determining the moment of invasion of the different tissues and the differential resistance of these to fungal attack.

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References

Adaskaveg, J.E., Blanchette, R.A., Gilbertson, R.L., 1991. Decay of date palm wood by white-rot and brown-rot fungi. Canadian Journal of Botany 69, 615–629.

Akin, D.E., Rigsby, L.L., Sethuraman, A., Morrison, W.H., Gamble, G.R., Eriksson, K.E.L., 1995. Alterations in structure, chemistry and biodegradability of grass lignocellulose treated with the white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus*. Applied and Environmental Microbiology 61, 1591–1598.

Alter, H., 1991. The future course of solid waste management in the U.S. Waste Management and Research 3, 3–20.

Asiegbu, F.O., Paterson, A., Smith, J.E., 1996. The effects of co-fungal cultures and supplementation with carbohydrate adjuncts on lignin biodegradation and substrate digestibility. Word Journal of Microbiology and Biotechnology 12, 273–279.

Barrasa, J.M., Camarero, S., Martínez, A.T., Ruel, K., 1995. Ultrastructural aspects of wheat straw degradation by *Phanerochaete chrysosporium* and *Trametes versicolor*. Applied Microbiology and Biotechnology 43, 766–770.

Blanchette, R.A., Otjen, L., Effland, M.J., Eslyn, W.E., 1985. Changes in structural and chemical components of wood delignified by fungi. Wood Science and Technology 19, 35–46.

Blanchette, R.A., Shaw, C.G., 1978a. Associations among bacteria, yeast and basidiomycetes during wood decay. Phytopathology 68, 631–637.

Blanchette, R.A., Shaw, C.G., 1978b. Management of forest residues for rapid decay. Canadian Journal of Botany 56, 2904–2909.

Bourbonnais, R., Paice, M.G., Reid, I.D., Lanthier, P., Yaguchi, M., 1995. Lignin oxidation by laccase isozymes from *Trametes versicolor* and role of the mediator 2,2′-azino-*bis* (ethylbenzothiazoline-6-sulfonic acid) in kraft lignin depolimerization. Applied and Environmental Microbiology 61, 1876–1880.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of micrograms quantities of protein utilizing the principles of protein-dye binding. Analytical Biochemistry 72, 248–254.

Braga, E.J., Uhart, M., Diorio, L.A., Forchiassin, F., 2002. Effect of co-fungal cultures on degradation of lignocellulose wastes. Revista Argentina de Microbiología 34, 39–44.

Chefetz, B., Hatcher, P.G., Hadar, Y., Chen, Y., 1996. Chemical and biological characterization of organic matter during composting of municipal solid waste. Journal of Environmental Quality 25, 776–785.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564–582.

D'Ambrogio, A., 1986. Manual de Técnicas en Histología Vegetal. Editorial Hemisferio Sur, Buenos Aires.

Davis, M.F., Schroeder, H.R., Maciel, G.E., 1994. Solid state ^{13}C nuclear magnetic resonance studies of wood decay. 1. White rot decay of Colorado blue spruce. Holzforschung 48, 99–105.

Díaz, M.J., Madejón, E., López, F., López, R., Cabrera, F., 2002. Optimization of the rate vinasse/grape marc for co-composting process. Process Biochemistry 37, 1143–1150.

Effland, M.J., 1997. Modified procedure to determine acid-insoluble lignin in wood and pulp. Tappi 60, 133–143.

Eriksson, K.E., 1988. Microbial delignification. Basics, potentials and applications. In: Aubert, J.P., Beguin, P., Millet, J. (Eds.), Biochemistry and Genetics of Cellulose Degradation. Academic Press, London, pp. 285–302.

Giusquiani, P.L., Pagliai, M., Gigliotti, G., Businelli, D., Benetti, A., 1995. Urban waste compost: effects on physical, chemical and biochemical soil properties. Journal of Environmental Quality 24, 175–182.

Gooday, G.W., 1995. The dynamics of hyphal growth. Mycological Research 99, 385–394.

Grabber, J.H., Ralph, J., Hatfield, R.D., Quideau, S., 1997. *p*-Hydroxyphenyl, guaiacyl and syringyl lignins have similar inhibitory effects on wall degradability. Journal of Agricultural and Food Chemistry 45, 2530–2532.

Haug, R.T., 1995. The Practical Handbook of Compost Engineering. Lewis Publishers, Boca Raton, 717 pp.

Hintikka, V., 1996. Substrate selection of some higher fungi in relation to their tolerances in aseptic cultures. Mycoscience 37, 49–53.

Johansson, M.B., Kögel, I., Zech, W., 1986. Changes in the lignin fraction of spruce and pine needle litter during decomposition as studied by some chemical methods. Soil Biology & Biochemistry 18, 611–619.

Migné, C., Prensier, G., Cornn, A., Grenet, E., 1996. Immunocytochemical localization of arabinoxylans in the cell wall of maize apical internode after microbial degradation in the rumen. Biology of the Cell 88, 137–144.

Nelson, N.J., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153, 375–380.

Nusbaumer, C., Job, D., Aragno, M., 1996. Etude de l'altération par les champignons de l'état physico-chimique des composés lignocellulosiques dans un processus naturel de compostage. Mycologia Helvetica 8, 51–67.

O'Brien, T.P., McCully, M.E., 1981. The Study of Plant Structure (Principles and Selected Methods). Termarccarphi, Melbourne.

Rihani, M., Botton, B., Villemin, G., El Abbouyi, A., 2001. Ultrastructural patterns of beech leaf degradation by *Sporotrichum pulverulentum*. European Journal of Soil Biology 37, 74–84.

Ruel, K., Ambert, K., Joseleau, J.P., 1994. Influence of the enzyme equipment of white-rot fungi on the patterns of wood degradation. FEMS Microbiology Reviews 13, 241–254.

Somogyi, M.J., 1952. Notes on sugar determination. Journal of Biological Chemistry 195, 19–23.