Chapter 15
Chaetomium as Potential Soft Rot Degrader of Woody and Papery Cultural Heritage

Ahmed M. Abdel-Azeem, Robert A. Blanchette, and Benjamin W. Held

15.1 Introduction

Wood deterioration is an essential process in the environment that recycles complex organic matter and is an integral component of life. These processes, however, also destroy historic wood that has been used as shelter, utility, and art resulting in a loss of valuable cultural properties from archaeological sites. Woods with natural resistance to microbial degradation were often used in ancient times for an application where wood was in contact with the ground, for shipbuilding and for other uses (Meiggs 1982). These extractive-rich woods helped to preserve the wood and resist microbial attack but even the most resistant woods are not immune from decomposition. Wood that persists for long periods of time is usually protected by an environment that limits microbial activity. These special conditions may allow wood to survive centuries or even thousands of years, but even in the most extreme environments, some physical and chemical modification of wood from biodeterioration takes place. What type of deterioration occurs and how these processes impact the wood are important questions that need consideration if wooden cultural properties are to be studied and properly preserved. Since there are relatively few wooden objects surviving from past civilizations, they are extremely valuable resources that deserve careful attention.

Chaetomium is a genus of filamentous fungi (phylum Ascomycota, class Sordariomycetes) encompassing species that typically possess densely setose, ovoid to pyriform ostiolate ascomata, clavate asci, and pigmented, one-celled ascospores (Samson et al. 2000). Species of *Chaetomium* are important in the decomposition of cellulose-rich materials.

A. M. Abdel-Azeem (✉)
Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt
e-mail: Ahmed_abdelazeem@science.suez.edu.eg

R. A. Blanchette · B. W. Held
Department of Plant Pathology, University of Minnesota,
St. Paul, Minnesota, United States of America

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In this chapter we will discuss the different ways of wood degradation caused by fungi and also describe specific degradation found in archaeological wood from a variety of different terrestrial with special reference to genus *Chaetomium*.

15.2 Fungi in the Museum Environment

Contamination of pieces of art presented in exhibition rooms or stored in depots and their spoilage by fungi is not exceptional but rather frequent in old and newly built museums (Allsopp et al. 2004; Nittèrus 2000; Capitelli et al. 2009; Mesquita et al. 2009; Pangallo et al. 2007; Koestler et al. 2003). It is well known to mycologists that fungi are able to inhabit, to alter, and to degrade all types of organic and inorganic materials. However, most conservators and museum curators are not aware of this enormous deteriorative potential.

Historically, pieces of art were made of all types of organic materials, and these materials are again used for an authentic restoration or conservation of the objects in recent times: Paint was made of mineral pigments bound with organic binders such as egg yolk, casein, linseed, poppy seed, hempseed oil, Chinese wood oil, or different resins. Linen canvas clamped on wooden frames serves as painting ground and was often primed with rabbit skin glue before painting. Gold leaf on precious wooden or stucco frames was applied using organic glues, linseed or turpentine oil. Historic glues were based on cellulose or rabbit skin. Sculptures and other art objects carry décors made of textiles, leather, straw, clay, natural hair, or feathers. The most precious documents of humankind are books and scrolls made of paper, papyrus, and parchment. Because of the tremendous diversity of exo-enzymes produced by fungi – cellulases, glucanases, laccases, phenolases, keratinases, monooxygenases, and many more – and their remarkable ability to grow at low aw values, the preservation of museum objects is inevitably connected with prevention of mold, monitoring of fungi, and treatment of fungi on contaminated objects.

A compilation of fungi frequently occurring on paper, paintings, and other materials in museum is given in Table 15.1 after Sterflinger (2010). The data are based on more than 20 studies carried out in Austrian museums since 2000 and on data collected from different case studies in the literature. Most fungi playing a role in the deterioration of cultural heritage phylogenetically belong to Euascomycetes; hemiascomycetes – yeasts – are rarely isolated from art objects. Teleomorphs are rarely found and the only teleomorph genera that frequently occur are *Chaetomium* – mostly on paper, wood, and feathers – and *Eurotium*, in environments with low aw values. Occurrence of basidiomycetes is restricted to wood degradation in churches or other protected historical monuments. Zygomycetes are frequently isolated from pieces of art but in most cases they can be regarded as transients not being really established on the objects.

Fungal growth on objects of cultural heritage often causes a serious aesthetical spoiling due to colony formation and fungal pigments (Sterflinger et al. 1999). Moreover, fungi degrade materials and thus affect objects substantially: the

Table 15.1 Most recovered fungi from museums and on materials objects of arts[a,b,c,d,e]

Substrate	Genus
Paintings: oil, water color, acrylic	*Alternaria sp., Aspergillus flavus, Aspergillus* sect. *niger, A. sydowii, A. versicolor, Aureobasidium pullulans, Chaetomium funicola, Cladosporium herbarum, C. cladosporioides, Eurotium chevalieri, E. rubrum, Fusarium* sp., *Mucor* sp., *Penicillium chrysogenum, P. citrinum, P. decumbens*, and many other species of the genus
Paper (laid paper, wood pulp paper) and cellulose textiles (cotton, linen)	*Alternaria* sp., *Aspergillus clavatus, A. flavus, A. glaucus, A. terreus, A. repens, A. ruber, A. fumigatus, A. ochraceus, A. nidulans, Aspergillus sect. niger, Botrytis cinerea, Chaetomium globosum, C. elatum, C. indicum, Eurotium amstelodami, Fusarium* sp., *Mucor* sp., *Paecilomyces variotii, Penicillium chrysogenum, P. funiculosum, P. purpurogenum, P. rubrum, P. variabile, P. spinulosum, P. fellutatum, P. frequentans, P. citrinum, Pichia guilliermondii, Rhizopus oryzae, Stachybotrys chartarum, Toxicocladosporium irritans, Trichoderma harzianum, T. viride, Stemphylium* sp., *Ulocladium* sp.
Parchment	*Cladosporium cladosporioides, Epicoccum nigrum, Phlebiopsis gigantea, Penicillium chrysogenum, Thanatephorus cucumeris*
Keratinous substrates (leather, wool, feathers, fur, hair)	*Absidia glauca, A. cylindrospora, A. spinosa, Acremonium* sp., *Alternaria alternata, Aspergillus sydowii, A. candidus, A. clavatus, A. carneus, A. foetidus, A. flavus, A. fumigatus*, and many other species of the genus *Arthroderma* sp., *Aureobasidium pullulans, Chaetomium globosum, Chrysosporium* sp., *Coniosporium* sp., *Cladosporium cladosporioides, Cunninghamella echinulata, C. elegans, Epicoccum nigrum, Emericella* sp., *Geotrichum candidum, Mucor* sp., *Penicillium brevicompactum, Penicillium chrysogenum,* and many other species of the genus, *Phoma medicaginis, Scopulariopsis* sp., *Stachybotrys chartarum, Trichophyton* sp., *Rhizopus* sp.
Archeological findings: bones, ceramics	Archaeological findings often carry a large load of spores, in case of contamination the diversity on the objects reflects the diversity of the respective soil

The identification of the fungi was carried out based on morphology and/or sequencing of the ITS1, 5.8 S, ITS2 region with subsequent homology search using the BLAST algorithm [http://www.ncbi.nlm.nih.gov/BLAST/]

[a]Unpublished data Sterflinger/ACBR

[b]Mesquita et al. (2009)

[c]Meier and Petersen (2006)

[d]Blyskal (2009)

[e]Pangallo et al. (2009)

enzymatic degradation of organic binders causes reduction or even loss of paint layers. Fungi penetrate cracks and migrate underneath paint layers thus causing detachment. In paper conservation fungi are a special problem due to their ability to excrete cellulases. Lignin-degrading fungi are rarely observed in indoor environments, but considerable damage can be caused by the cellulose degraders *Serpula lacrymans* or *Coniophora puteana* in churches and other objects of cultural heritage if wooden altars or the roof structures are attacked. Also originals and museum reconstructions of historical buildings are considerably damaged by *S. lacrymans* (Bech-Andersen and Elborne 2004).

The development of fungi in museums is to a large extent determined by the indoor climate, the amount of available nutrients – from the atmosphere and from the materials themselves – and also the cleaning intervals in the museum. The indoor climate as indicated by temperature, relative humidity, and specific humidity is the most important factor for fungal growth. It is also closely related to the building's physical properties, especially the thermal insulation and the tendency to generate condensate from warm indoor air on cold walls of the building envelope (Camuffo 1998). Depending on the climate in the museum or storage rooms, the fungal diversity is restricted to few xerophilic and xerotolerant species such as *Eurotium* sp., *Aspergillus* sp., or *Wallemia* sp. Only in storage rooms where the humidity is raised to more than 70% for a period of several weeks or month is a high fungal diversity able to establish. The climate ranges allowing fungal spores to germinate and that restrict the growth of the fungal mycelium are shown in the isopleth systems by Sedlbauer and Krus (2003). The authors also show that hygroscopic materials support the growth of fungi at low relative humidity and that the water demand depends on the biodegradability of the substrate. The objects influence the development of the fungal community by their chemical composition and biodegradability for species with different exo-enzymes.

In museums the range of 55% Relative Humidity (RH) is generally regarded as the borderline for fungal growth and thus climate control is adjusted below this value. In fact, fungi that are able to survive at a relative humidity of 55% are rare and restricted to extreme environments such as hot and cold deserts. So why do mesophilic hyphomycetes frequently occur in museums? All museums in the world measure temperature and humidity in storage and exhibition rooms by means of modern data logger, data writers, or simple hygro- and thermometers. However, the way and location of climate measurements are often insufficient to reflect the real climate and to detect different climatic zones in the building. In his book on microclimate in museums, Camuffo (1998) illustrates the complexity of climate monitoring that cannot just be monitored by a single data logger in the middle of a museum room. The influence of airstream through doors, warming by sunlight, and daily changes of temperature gradients as well as the isolation and exposition of the building envelope have to be considered as important factors. In fact, fungal growth mostly happens between shelves with little aeration or near to walls with temperatures below the dew point. Micro-niches are often also created by wrapping of single objects into plastic foils or extremely tight boxes not allowing an exchange of air and vapor.

The mycobiota in museums is also influenced by the atmospheric particulate matter carrying carbonates, minerals, and others. Gysels et al. (2004) have shown in a study on the Royal Museum of Fine Art in Antwerp that the indoor aerosols were largely determined by the outdoor atmosphere and the outdoor sources of organic and inorganic pollutants. Fungi are well able to degrade different types of organic pollutants including polycyclic aromatic hydrocarbons (PAHs). Therefore, the fungal diversity on monuments in an urban environment was found to be much higher than in a rural environment of the climatic zone (Sterflinger and Prillinger 2001).

As a common species of *Chaetomium*, *Chaetomium globosum* can utilize a variety of carbohydrates as sources of carbon (Table 15.2) for the listing of the sources based on testing conducted at Health Canada.

Table 15.2 Carbohydrate utilization used for taxonomic identification of *C. globosum* strain ATCC 6205, based on the RapID™ YEAST PLUS system

Carbohydrate[a]	Result
Glucose	–
Maltose	–
Sucrose	–
Trehalose	–
Raffinose	–
Fatty acid ester	–
p-Nitrophenyl-N-acetyl-β,D-galactosaminide	+
p-Nitrophenyl-α,D-glucoside	–
p-Nitrophenyl-β,D-glucoside	+
o-Nitrophenyl-β,D-galactoside	–
p-Nitrophenyl-α,D-galactoside	+
p-Nitrophenyl-β,D-fucoside	–
p-Nitrophenyl phosphate	+
p-Nitrophenyl phosphorylcholine	–
Urea	+
Proline β-naphthylamide	+
Histidine β-naphthylamide	+
Leucyl-glycine β-naphthylamide	–

[a]The Thermo Scientific™ RapID™ YEAST PLUS System was used to evaluate sugar consumption of *C. globosum* strain ATCC 6205. *C. globosum* strain ATCC 6205 was inoculated into wells containing various carbon sources and incubated at 28 °C. The wells were evaluated after 48 hours
"+" means that the test result was positive and "–" means that the test result was negative

Prokhorov and Linnik (2011) reported *C. globosum* growth studies on wort agar, or mineral medium, and either glucose, saccharose, mannite, lactose, amylum or cellulose as carbon sources. For all carbon sources, fungal colony sizes increased on all media beyond three days and ascocarps formed on all media. In the environment, *C. globosum* uses natural sources such as cellulose in straw (Harper and Lynch 1985), the wood of the European beech (*Fagus sylvatica*) (Mohtashamipur and Norpoth 1990), heartwood and sapwood from the brazilwood tree (*Caesalpinia echinata*) (Silva et al. 2007), and fungal cell wall (*Pythium ultimum*) (Inglis and Kawchuk 2002). It is also capable of utilizing keratin in feathers (Kaul and Sumbali 1999) and collagen in leather (Strzelczyk et al. 1989).

15.3 Structural and Chemical Features of Wood

Wood consists of an orderly arrangement of cells with walls composed of varying amounts of cellulose, hemicellulose, and lignin. The great diversity of woody plants is reflected in the varied morphology and chemical composition of their wood. Typically, two general groups, *hardwoods* (angiosperms) and *softwoods* (gymno-

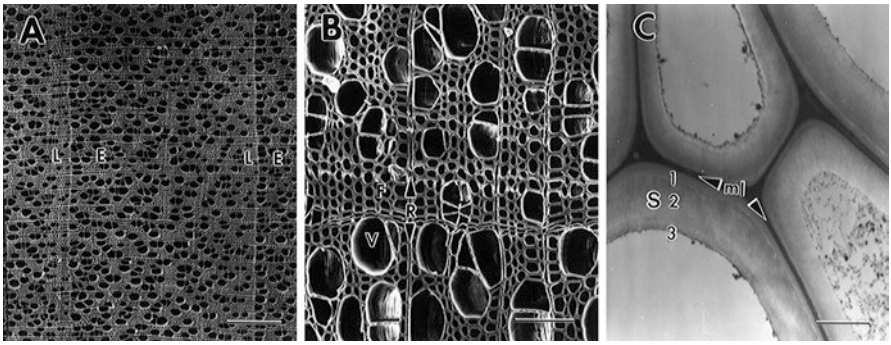

Fig. 15.1 Cell structure of an angiosperm. (**a** and **b**) Sections of a diffuse porous hardwood showing earlywood (E) and latewood (L). The wood consists of vessels (V), fibers (F), and ray parenchyma cells (R). (**c**) Cell walls with secondary wall layers (S1, S2, and S3) and middle lamellae (ml). Transverse sections. A and B SEM, C TEM. Bar = 500 μm in A, 100 μm in B, and 2 μm in C (Blanchette 2000)

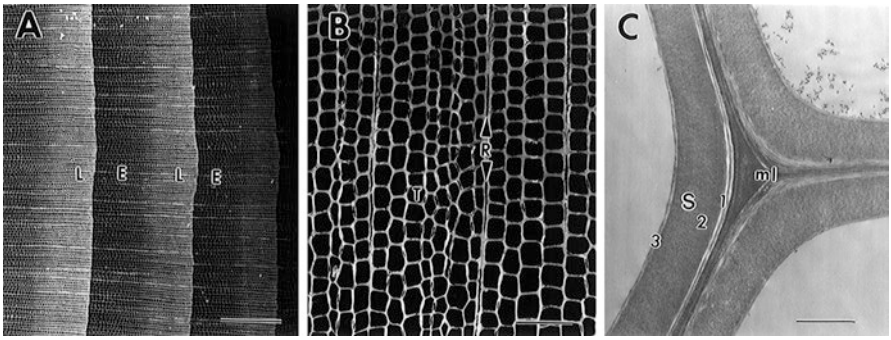

Fig. 15.2 Cell structure of a gymnosperm. (**a** and **b**) Thin-walled earlywood or springwood (E) and thick-walled latewood (L) tracheids. (**b**) Zone of earlywood showing tracheids (T) and ray parenchyma cells (R). (**c**) A group of tracheid cell walls showing secondary wall layers (S1, S2, and S3) and middle lamella (ml). Transverse sections. A and B SEM, C TEM. Bar = 500 μm in A, 100 μm in B, and 2 μm in C (Blanchette 2000)

sperms), can be easily separated. Hardwoods have pores or vessel elements that occur among fiber and parenchyma cells (Fig. 15.1). Cellulose content ranges from 40% to 50% with 15–25% lignin and 15–25% hemicellulose. The remaining components consist of various extracellular compounds.

Softwoods are composed of overlapping tracheids, connected by bordered pit apertures, and parenchyma cells and, in some cases, resin canals (Fig. 15.2). Greater concentrations of lignin, about 5–10% more than hardwoods, are found in softwoods and about the same amount of cellulose 40–50%. Less hemicellulose may be found in softwoods than hardwoods. The chemical composition of softwoods is also different from hardwoods with different types of lignin (primarily guaiacyl propane units), hemicelluloses (mannose is the most common constituent), and wood extractives (different terpenes, fatty acids, etc.).

15.4 Degradation of Wood by Fungi

Microbes that degrade wood produce extracellular enzymes that break down the woody cell wall. Growth characteristics of the microorganism in wood and the type of degradative system produced results in different decay patterns being produced (Blanchette 1995, 1998). Depending on the type of decay, different physical, chemical, and morphological changes occur in wood. These decay processes have been well characterized and provide useful insights to elucidate deterioration in archaeological woods (Table 15.3). A review of decay patterns produced by different fungi suggests that three categories can be used to separate the types of decay produced in wood. Names for these categories are based on visual characteristics of the advanced decay.

Two major groups of decay produced by fungi taxonomically classified in the subdivision *Basidiomycota* are *white* and *brown* rot fungi. *White rot fungi* can degrade all cell wall components, including lignin. They often cause a bleaching of normal wood coloration. Their ability to metabolize large amounts of lignin in wood is unique among microorganisms. The thousands of species that cause white rots are a heterogeneous group that may degrade greater or lesser amounts of a specific cell wall component. Some species preferentially remove lignin from wood leaving pockets of white, degraded cells that consist entirely of cellulose, while others degrade lignin and cellulose simultaneously (Blanchette 1991). Degradation is usually localized to cells colonized by fungal hyphae and substantial amounts of undecayed wood remains. A progressive erosion of the cell wall occurs when components are degraded simultaneously (Fig. 15.3) or a diffuse attack of lignin may occur by species that preferentially remove lignin (Blanchette 1991). Strength losses are not significant until late stages of decay (Cowling 1961; Zabel and Morrell 1992).

Table 15.3 Microbes that cause wood degradation (Blanchette 2000)

Microorganism	Wood components utilized	Decay characteristics
Fungi		
White-rot	All cell wall components, some species preferentially attack lignin	Progressive erosion of all cell wall layers. Middle lamella is degraded
Brown rot	Carbohydrates, some lignin modification	Diffuse depolymerization of cellulose
Soft-rot	Carbohydrates, some lignin modification	Type 1 – cavities form in secondary wall Type 2 – progressive erosion of secondary walls, but middle lamella is not degraded
Bacteria		
Erosion	Carbohydrates, extent of lignin modification not known	Erosion troughs leaving large quantities of residual wall material
Tunneling	Carbohydrates and some lignin	Minute tunnels in secondary walls and middle lamella
Cavitation	Carbohydrates, extent of lignin modification not known	Cavities form in secondary wall leaving residual wall material
Scavengers	Primary organisms: pit membranes but no cell wall decay. Secondary organisms: utilizes modified cell wall components	Primary organisms penetrate pits and degrade wood extractives. Secondary bacteria scavenge altered wood components

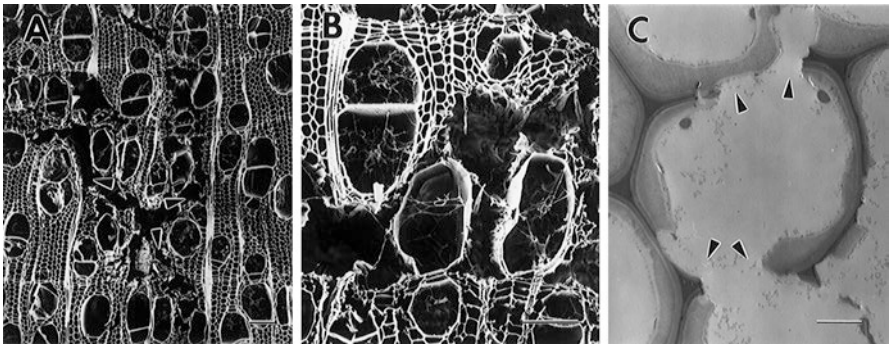

Fig. 15.3 Nonselective attack of wood by a white rot fungus. (**a** and **b**) All cell wall components are degraded resulting in voids within the degraded wood. (**c**) A progressive attack of all cell wall components causing a localized erosion of the secondary wall layers and middle lamellae (arrowheads). Transverse sections. A and B SEM, C TEM. Bar = 100 μm in A and B, 5 μm in C (Blanchette 2000)

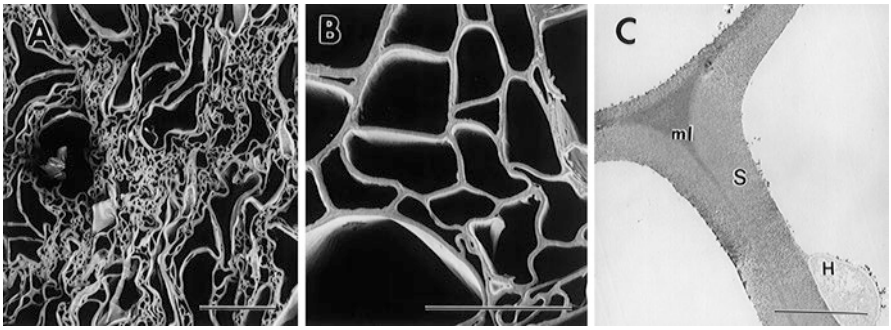

Fig. 15.4 Decay of wood by brown rot fungi. (**a**) Degradation of cellulose in woody cell walls leaves a residual network of lignin. Cell walls collapse and appear distorted. (**b**) Degraded cells showing walls that are porous and fragile. (**c**) Ultrastructure of a cell consisting primarily of residual lignin that has little binding strength and is loosely held together. H hypha, S secondary wall, ml middle lamellae (Blanchette 2000)

White rot fungi are common parasites of heartwood in living trees and are aggressive decomposers of woody debris in forest ecosystems (Blanchette 1991; Rayner and Boddy 1988).

Brown rot fungi depolymerase cellulose rapidly during incipient stages of wood colonization. Considerable losses in wood strength occur very early in the decay process, often before decay characteristics are visually evident (Wilcox 1968). Cell wall carbohydrates are degraded extensively during decay leaving a modified, lignin-rich substrate (Fig. 15.4). The residual wood is brown and often cracks into cubical pieces when dry. Brown rot fungi commonly cause decay of timber in buildings, and these fungi have had serious impact on ancient and historic buildings (Jennings and Bravery 1991). One of the most destructive brown rot fungi is *Serpula lacrymans* (previously called *Merulius lacrymans*). This fungus has become well adapted to attacking timber in service and can spread rapidly on wood and traverse nonnutritional surfaces. Aerial mycelia differentiate into thick strands that allow the

fungus to invade new substrates. The mycelial strands also act to transport moisture and nutrients to considerable distances (Jennings 1991). Commonly, this type of decay has been referred to as dry rot. This term, apparently first used to describe any deterioration of dead wood or wood in service (Britton 1875), is misleading because moisture must be present for the decay to occur.

15.5 Wood Cell Wall Degradation by Soft Rot Fungi

Soft rot is caused by a diverse range of microfungi from the ascomycetes (teleomorphic and anamorphic) and has ubiquitous distribution throughout the world. Soft rot can have severe economic consequences since several of the species involved attack wooden constructions including preservative-treated wood under terrestrial (Fig. 15.5a) and aquatic (marine and sweet water) situations (Gersonde and Kerner-Gang 1976; Leightley 1977; Leightley and Eaton 1978; Zabel et al. 1985; Daniel and Nilsson 1989). For example, soft rot has been documented as one of the major forms of attack of preservative-treated (i.e., with waterborne preservatives like copper–chrome–arsenic and creosote) utility poles (Gersonde and Kerner-Gang 1976; Henningsson and Nilsson 1976; Zabel et al. 1985). Visually, soft-rotted wood becomes gray and even black in advanced stages of decay due to colonization by fungi hyphae in the wood. The surface of soft-rotted wood also tends to crack in a similar way as brown rot. The term "soft rot" was originally used to describe the decay from waterlogged wood, which was soft to touch (Savory 1954). However, this definition is not correct, and wood from terrestrial environments can be very heavily degraded but still remain hard as, for example, seen with utility poles. When such poles are strained, typical brash fractures are produced in contrast to that of brown rot, which produces brittle fractures due to depolymerization of the cellulose. Thus, soft rot can give rise to significant reductions in the strength of wood at comparable small weight losses although not to the level of brown rot.

Soft rot decay of wood is strongly affected by both lignin type and concentration in contrast to brown rot fungi and more significantly than white rot decay. Thus, softwoods (e.g., spruce and pine) show greater resistance to attack than hardwoods especially low lignin hardwoods (e.g., aspen and birch beech). In addition, at the cellular level in hardwoods, the variation in microdistribution of syringyl and guaiacyl lignin that occurs between cell types (vessels/fibers/ parenchyma) very often creates variations in decay morphology (Daniel and Nilsson 1998).

15.5.1 Morphological Aspects of Cell Wall Decay by Soft Rot Fungi

Soft rot produces two distinct forms of attack of wood decay known as Type I in which characteristic cavities are produced in cell walls and Type II where hyphae localized in the cell lumina cause cell wall erosion (i.e., cell wall thinning) (Figs. 15.5, 15.6, and 15.8) (Table 15.4). In particular, cavity formation is very

Fig. 15.5 Soft rot decay of wood. (**a**) Utility pole with outer regions degraded by soft rot. (**b**, c) Soft rot Type I with cavities orientated along the cellulose microfibrils in the S2 layer. (**d**, **e**) Transverse sections of pine at early (cavities) and late stages of soft rot Type I. At late stages, only the middle lamellae and S3 layers remain in softwoods. Transverse sections of pine (**f**) and birch (**g**) showing soft rot cell wall erosion (Type II). Ultimately, only the middle lamellae remain (Daniel 2016)

diagnostic for soft rot decay although many of the soft rot fungi cause both decay types simultaneously, sometimes in the same cells. A few species only cause Type II attack (Nilsson 1973). Type I decay results in the formation of characteristic cavities produced by hyphae within secondary cell walls that align along the cellulose microfibrils (Figs 15.5b–d), their presence best observed using longitudinal sections under polarized light (Fig. 15.5c). Type I decay is initiated by specialized microhyphae (~0.5 mm) or fine penetration hyphae that grow from the cell lumen into the S2 layer of wood cell walls (Leightley 1977; Crossley 1979; Hale and Eaton 1985a, b; Daniel and Nilsson 1989).

Such hyphae can penetrate directly through adjacent cells without forming a cavity and expand again to the native size in the adjacent lumen (i.e., rather like blue-stain fungi). It is only when the hypha reorientates itself in the S2 layer by forming either a T-branch or L-bend that cavity formation is initiated. When orientated along the

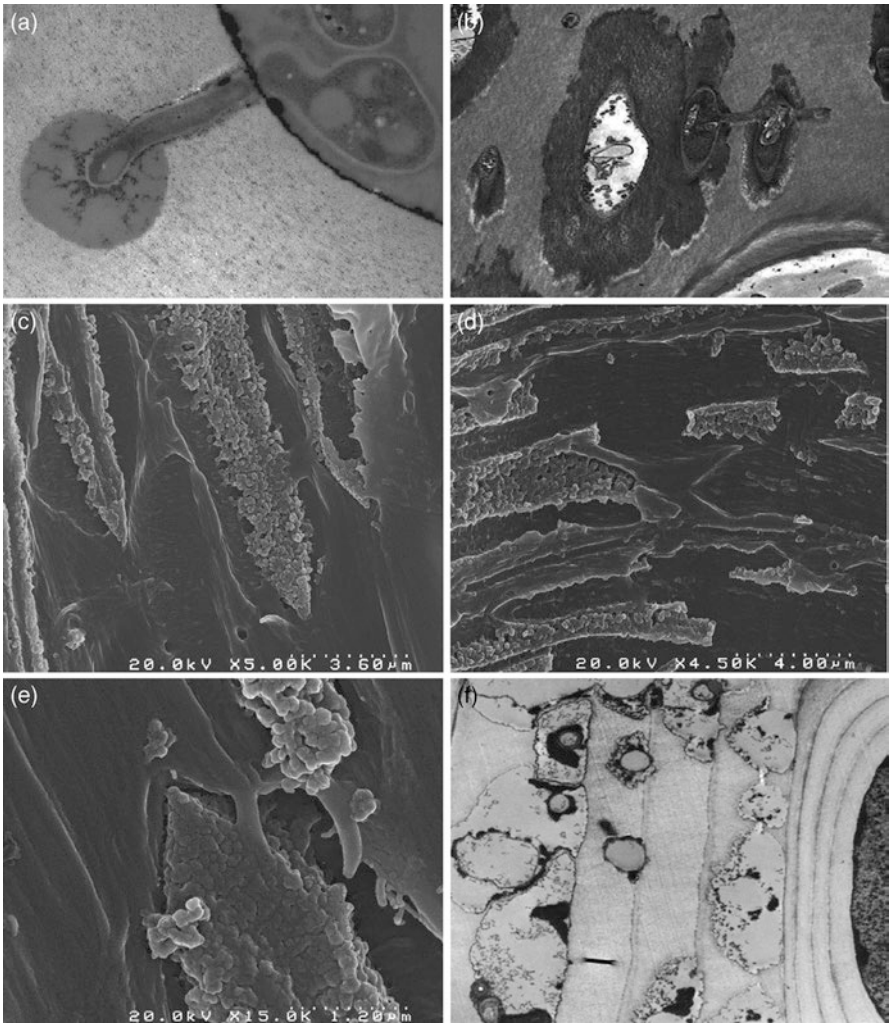

Fig. 15.6 Soft rot Type I. TEM sections through birch (**a**), pine (**b**), and *H. foetidum* (**f**) wood degraded by soft rot Type I. Sites of Tbranching, multiple Tbranching, and cavity formation in the S2 layer (**a**, **b**). (**c**–**e**) SEM images of soft rot cavities in S2 (**c**, **e**) and S1 layers (**d**). Hyphae are covered in melanin deposits and lignin breakdown products. Multilayered fiber of *H. foetidum* showing effect of lignin on soft rot attack with halfmoon-shaped cavities produced against thin concentric layers containing high lignin in the S2 layer (**f**) (Daniel (2016)

cellulose microfibrils in the cell wall, the hypha stops growing and produces a cavity (Hale and Eaton 1985a, b; Daniel and Nilsson 1998). When the cavity has enlarged, growth can be resumed by the hypha either from one end (L-bending) or from both ends (T-branching) simultaneously. After the cavities have enlarged, the process repeats itself and chains of cavities are produced (Figs 15.5b, c). The process of cavity formation has been very well documented and the size and shape of the cavities are known to vary with fungal species and wood type (Daniel and Nilsson 1998). T-branching is not limited to single branches and soft rot fungi are frequently known

Table 15.4 Classification of morphological effects of fungal attack of wood cell walls (Daniel 2016)

Decay type	Morphological changes of wood cell walls	Cell wall components attacked	Taxonomic grouping	Typical examples
White rot (simultaneous)	Hyphal bore holes enlarge Cell wall thinning from lumen Middle lamellae degraded Development of cavities in some species	Cellulose, lignin, hemicelluloses Extractives	Basidiomycetes Higher ascomycetes	*T. versicolor*[a] *Heterobasidium annosus* *Xylaria polymorpha*[b] *Daldinia concentrica*[b]
White rot (preferential)	Hyphal bore holes enlarge Cell wall attack from lumen	Hemicelluloses, lignin Extractives	Basidiomycetes	*Ceriporiopsis subvermispora* *Heterobasidion annosum* *Phl. radiata Cel 26*
	Middle lamellae degraded Fiber defibration and separation			
Brown rot	Rapid attack of cell walls Cell wall attack from lumen	Depolymerization of cellulose, hemicelluloses; lignin modified	Basidiomycetes	*C. puteana*[a] *Ol.* (*Postia*) *placenta*[a]
Soft rot Type I	Hyphal bore holes remain small Longitudinal cavities Middle lamellae remain	Cellulose, hemicelluloses lignin modified/ degraded	Ascomycetes Fungi imperfecti	*Chaetomium globosum*[a] *Phialophora mutabilis*
Soft rot Type I, *diffuse type*	Hyphal bore holes remain small Coalescence of longitudinal cavities	Cellulose, hemicelluloses lignin modified/ degraded	Ascomycetes	*Phi. dimorphospora* *C. globosum*
	Middle lamellae remain		Fungi imperfecti	*Bispora betulina*
Soft rot Type II	Hyphal bore holes remain small; cell wall thinning from lumen; middle lamella remains	Cellulose, hemicelluloses lignin modified/ degraded	Ascomycetes Fungi imperfecti	*C. globosum* *Phialop. mutabilis*
Blue stain fungi	Fine bore holes Small cavities/erosion troughs	Primarily nonlignified cells (ray parenchyma), extractives	Ascomycetes Fungi imperfecti	*Ophiostoma piceae* *L. theobromae*
Mold fungi	Growth in surface regions	Soluble sugars, extractives	Ascomycetes Fungi imperfecti Zygomycetes	*C. globosum* *Penicillium brevicompactum* *Aspergillus versicolor* *Rhizopus* spp.

[a]*Frequently used as test fungi for assessing new types of wood protection – for example, preservatives, wood modification*

[b]*Higher ascomycetes cause white rot but are not known to degrade middle lamella regions*

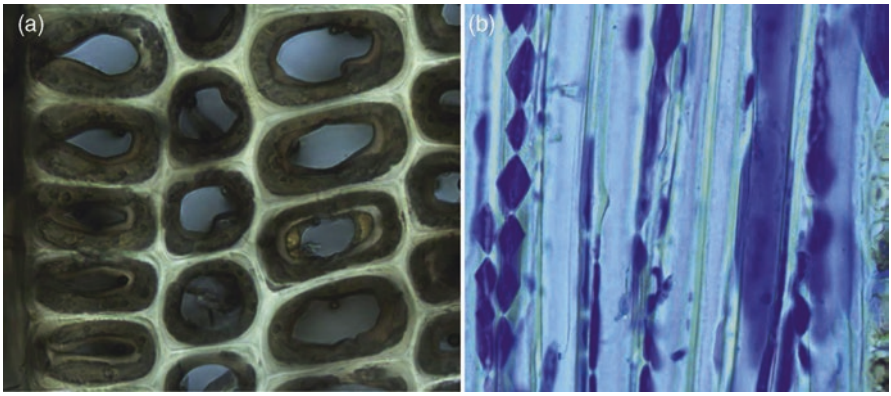

Fig. 15.7 Diffuse soft rot Type I decay of pine and birchwood by *Phi. dimorphospora*. (**a**) Soft rot hyphae within the secondary cell walls (S2) with diffuse widespread decay not restricted to cavities. (**b**) Cavities (stained blue to right of photo) are often very large and most of the secondary wall degraded. Note: Subpart (**a**) is not stained, but colored black through melanized hyphae and melanin secretions (Daniel 2016)

to develop multi-T-branching in cell walls (Fig. 15.7b) as observed with some white rot basidiomycetes. The fact that cavity formation always follows the orientation of the cellulose microfibrils is shown by the circular orientation of hyphae that invade pit chamber walls and between pits (Khalili et al. 2000). Cavities are most often observed first in the thick latewood cells (Fig. 15.5d, e) of wood spreading thereafter to earlywood. The relationship here is thought to be related to the size of the hyphae as cavities are rarely observed in the S1 and never in the S3 layer (i.e., it is too thin and has high lignin in softwoods), although in advanced stages of decay the S1 and S3 are degraded through hyphal attack from the adjoining S2 layer.

The effect of lignin content and lignin type is shown at the cellular level in advanced stages of attack where only the high lignin middle lamellae remain in hard-and softwoods (Fig. 15.5e). In addition, the hyphae and cavities often have darkly staining materials closely associated consisting of lignin remnants and melanin, the latter directly attached to hyphae or extracellular within the cavities (Figs 15.5e, 15.6c–f, and 15.7a). A similar effect can also be noted in fibers showing very thin concentric layers containing higher lignin levels than the surrounding cell wall (e.g., *Homalium foetidum*; Fig. 15.6f). Here, "half-moon" cavities are produced with flat axis orientated along the more lignified layer. That soft rot fungi need to orientate along the cellulose to cause cavity formation suggests it is an adaptation to overcome the effect of lignin coating the cellulose at the macromolecular level. Since the effect is shown in soft and hardwoods with different lignin types and content, it is suggested that it is the cellulose orientation that is most important. This is shown quite easily by slight delignification of wood, which enhances not only its susceptibility to soft rot but changes the nature of the decay process by modification or loss of cavity formation.

Type II soft rot is very similar to simultaneous white rot attack and can result in a complete removal of the secondary cell walls but in contrast the middle lamellae remain (Fig. 15.5f, g). This decay type is very frequently observed in low-lignin hardwood (e.g., aspen and birch) especially under high moisture situations.

A third decay form known as “diffuse cavity formation” has been described in which attack is similar to Type I during the initial stages but after cavity formation solubilization of the polysaccharides is more widespread and diffuse in the S2 layer and more like brown rot attack (Fig. 15.7a, b). The decay type is best observed in low-lignin-containing hardwoods (e.g., birch) although it does occur in softwoods. Typical soft rot species producing this decay pattern include *Phialocephala dimorphospora* (Anagnost et al. 1994). Blanchette (2000) concluded that Type 1 is characterized by longitudinal cavities formed within the secondary wall of wood cells and Type 2 used to describe an erosion of the entire secondary wall (Fig. 15.8).

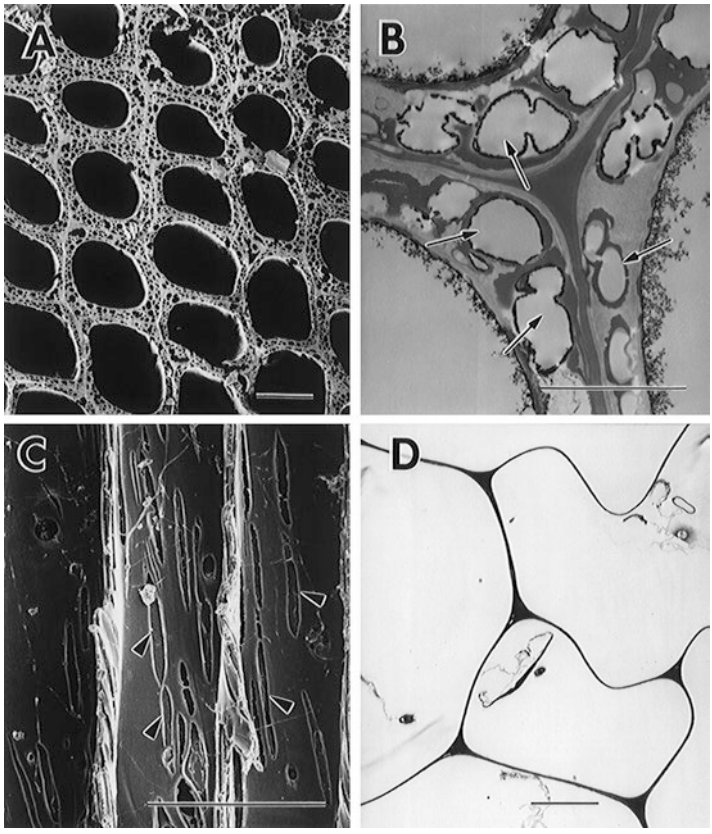

Fig. 15.8 Soft rot attack of wood. (**a**–**c**). Type I attack forms chains of cavities within the secondary wall (arrows). In advanced stages of decay, cell walls contain numerous cavities that often coalesce together (arrows). In some cells, the chains of cavities are visible from the cell lumina (arrowheads) where holes in the wall have been exposed. (**d**) Type II form of attack showing an erosion of the secondary wall but no degradation of the middle lamella. In advanced decay, the secondary walls are completely degraded and only the middle lamellae remain. A, B, and D transverse sections, C radial section. A and C SEM, B and D TEM. Bar = 50 μm in A and C, 5 μm in B and D (Blanchette 2000)

15.6 Key to Identification of Wood Decays Based on Light Microscopic Features (Anagnost 1998)

la Erosion channels on the lumen surface present 2
b Erosion channels absent from the lumen surface 5
2a Erosion channels rounded with V-notches, hardwoods or softwoods 3
b Erosion channels angular with V-notches, in hardwoods only 4
3a Erosion appears as elongate channels beneath lumen hyphae; channels may be numerous; pit erosion is rounded or oval; bore holes and pit erosion are common; some cell separation may be evident; cavities in the S2 are rare; if present, cavities not in chains, penetrating bore hole is wider than hyphae ..... *White rot* (simultaneous)
b Cell separation is common; erosion channels are sometimes present ..... *White rot* (selective delignification)
4a Bore holes, if present, are smaller in diameter than penetrating hyphae; pit erosion is angular often forming diamond-shaped erosion or lacking ..... *Soft rot* (type 2)
b Erosion is accompanied by cavities within the cell wall; pit erosion is angular (diamond-shaped) or lacking ..... *Soft rot* (type 1 and 2)
5a Bore holes often lacking or rare (except in some cases; if present, bore hole diameters are the same as (early stages) or slightly wider than (later stages; up to 4*um*) associated hyphae); brown discoloration or shrinkage cracks may be evident; birefringence in polarized light may be lacking; loss of cell shape and wall thickness (incrosssection);rayparenchymadestroyedinearlystages……. *Brown rot*
b Bore holes initially smaller than hyphae. 6
6a Bore holes initially small, but enlarge to become much larger than penetrating hyphae; erosion channels may develop; cell separation sometimes evident; cavities in S2 are rare; if present, hyphae t- or I-branching (sometimes multiple branching occurs) to form very narrow cavities within the S2 layer which extend and widen; initial bore holes widen obviously.....*White rot*
b Bore holes remain smaller than penetrating hyphae 7
7a Cavities are present within the S2 layer arising from t- or I-branching from transverse bore holes 8
b Narrow bore holes may be present; hyphae in cell lumens, often primarily in ray cells; wood may be discolored ..... *Early decay, stain or mold*
8a In hardwoods and softwoods, hyphae form diamond-shaped cavities within the S2 1ayer ..... *Soft rot* (type 1 regular)
b In softwoods only, hyphae form individual cavities that develop into diffuse cavities within the S2 cell wall; cavities appear as patches of S2 destruction at later stages with differential interference contrast microscopy ..... *Soft rot* (type 1 diffuse)

15.7 Soft Rot Fungi: Enzymes Involved and Some Biochemical Aspects of Decay

Numerous studies have been conducted to understand the biochemical/chemical mechanisms involved in fungal decay of lignocellulose, the majority aimed at biotechnological goals rather than using the understanding to produce better protective measures of wood in service. In principle the majority of these studies have been carried out using liquid cultures of fungal monocultures together with lignocellulose in various forms (e.g., as particles, sawdust, or flakes), purified cellulose/hemicelluloses, or lignin monomers (e.g., synthetic/natural). While these studies give information on the types of enzymes that may be produced under various physiological conditions (e.g., temperature, pH, and shaking/static), it may not reflect the situation that occurs in wood substrates under native conditions, but rather the potential ability of the fungi involved. For example, few studies have actually been involved in measuring enzyme activities in wood undergoing decay because of the difficulties in extracting sufficient amounts of "active proteins" in order to carry out enzymatic assays. Notable exceptions include the studies by Daniel et al. (1992, 1994) on the extraction of lignin-degrading enzyme (LiP, MnP, laccase) produced by *Pha. chrysosporium*, *T. versicolor*, and *O. mucida* in birchwood. A further problem concerns the sensitivity of the enzyme assays, which may not be sufficiently sensitive to detect the minor amount of proteins that can be extracted even in highly degraded wood materials. For example, extraction of proteins from whiterotted wood in which profuse hyphae growth is often seen is easier than from either brown or softrotted wood. In recent years, a variety of genomic, transcriptome, and secretome analytical approaches have been developed allowing for profile overviews of the enzyme systems available or employed by various fungi when grown on purified wood components (e.g., cellulose) (Martinez et al. 2009). This approach gives a more in-depth view of the enzymes or enzyme systems potentially involved, and the changes in enzyme profiles over time during wood decay can be followed. Evidence for the upregulation of a gene does not however mean it is actually involved in decay but rather that it has a potential. Possibly one of the most important approaches although indirect for proving the involvement of enzymes at sites of wood decay is by using antibodies produced against the purified proteins in microscopy assays. The easiest approach is by indirect labeling and the use of secondary antibodies whereby the sites of primary antibody labeling of enzymes in situ are recognized by the secondary antibodies carrying a fluorescence or gold label, which can then be visualized using fluorescence/confocal microscopy or electron microscopy. These studies were very important in proving the extracellular secretion of enzymes involved in decay and their remote distribution from hyphae during the wood decay process (Daniel et al. 1989, 1990, 1994, b, 2007; Ruel et al. 1990; Srinivasan et al. 1995). A wide variety of enzymes may be directly involved in hydrolytic activities (e.g., cellulases, hemicellulases, and pectinases) or act as oxidases in the production of oxidants that indirectly affect wood components (e.g., OH radicals from H_2O_2, pyranose 2oxidase, and glucose 1oxidase). A further important characteristic concerns the nature of the enzymes and whether they are associated with extracellular matrix materials such as slime and other polysaccharidebased materials (Daniel 2014) (Fig. 15.8).

Compared with white and brown rot fungi, much less is known about the degradative enzyme systems produced by ascomycetes during soft rot attack of wood in which cavity and erosion decay occur. Cavity formation in hardwoods is easier as shown by the larger number of cavities formed in a fixed period of time. Currently, there are no indications that enzymatic systems used in cavity formation differ from those used in cell wall erosion. The most important requirement for soft rot cavity formation is the alignment of hyphae with the cellulose microfibrils, the alignment probably inducing the secretion of cell wall-degrading cellulases. This indicates that the enzyme systems involved are likely to be closely associated with the fungal hyphae and initially at least present on the hyphal surfaces (Fig. 15.6a–f). The fact that the bioconical cavities are formed by hyphae aligned with the cellulose microfibrils in wood cell walls and not by the thin penetration (i.e., bore hyphae) traversing cell walls also suggests involvement of different or the absence of enzyme systems. Studies indicate that most cellulolytic microfungi cause some erosion of hardwood cell walls even those essentially regarded as typical mold fungi (e.g., *Aspergillus*, *Penicillium*, *Trichoderma* spp.); however, their ability to cause the same attack on softwoods is greatly limited. Frequently, *Trichoderma* spp. are reported as a typical soft rot fungi of wood and thus used as an example for comparison with white and brown rot fungi. However, as indicated earlier, *Trichoderma* cannot be really classified as a true soft rot fungus as it has not been unequivocally confirmed to produce cavities in the secondary cell walls of softwoods.

Few studies have been conducted on the chemical changes in wood following soft rot attack. It is clear, however, that since soft rot fungi have an inability to degrade the middle lamella regions in wood, their ability to degrade lignin is more limited than that of white rot fungi, and thus an increase in the relative lignin content in wood due to preferential removal of polysaccharides is expected. Lignin degradation has been reported, but it seems related to the fungal species involved. Chemical analyses of lignin from beechwood degraded by soft rot fungi have shown a lower methoxyl content and greater acid solubility than in undegraded wood (Levi and Preston 1965). Recent studies on birchwood degraded by a range of fungi imperfecti and ascomycetes have shown decay characterized by lower lignin (Klason) loss compared to white rot and much lower alkali solubility compared with brown rot (Worrall et al. 1997). Lignin peroxidase has been isolated and purified from the ascomycete *Chrysonilia sitophila* (Durán et al. 1987; Rodríguez et al. 1997), although the fungus has not been shown to degrade wood. Similarly, a range of phenolic and lignin-related compounds have been shown degraded by soft rot fungi (Haider and Trojanowski 1975; Bugos et al. 1988), but this does not confirm they degrade lignin in wood or cause wood decay. A number of thermophilic ascomycetes (Machuca et al. 1998) and some heat-tolerant soft rot fungi like *Talaromyces thermophilus* and *Thielavia terrestris* are also weakly ligninolytic, but their true effect of wood is limited (Dix and Webster 1995). Observations show extensive removal of wood cell wall materials surrounding cavities produced in the secondary walls (S2, S1 layers) of both hard and softwoods in advanced stages (Figs 15.5d, e and 15.6a, b, f). However, in both wood species and depending on soft rot fungus, there are generally always large amounts of residual electron materials surrounding and attached to the hyphae (e.g., Fig. 15.6f) (Daniel and Nilsson 1989).

These materials represent partially degraded lignin remaining after preferential cellulose/hemicellulose removal as well as melanin.

The ability of soft fungi to preferentially remove carbohydrates and leave lignin in cavities and in the cell wall during erosion decay indicates the involvement of a diffusive cellulase/hemicellulase system. This is consistent with several large screening studies carried out on soft rot fungi from terrestrial, marine, and freshwater situations showing enzymatic "clearing" (i.e., decay) of ball mill cellulose/cellulose agar and hemicellulose (xylan) agar during growth of these microfungi (Nilsson 1973; Bucher et al. 2004; Duncan et al. 2006, 2008; Simonis et al. 2008). These enzyme–agar studies showed that clearing zones could occur a considerable distance from the fungal hyphae indicating a highly diffusible enzymatic system that varies with fungal species. The fact that cavity formation and erosion decay is a frequent form of decay in aquatic situations emphasizes not only the success of this form of decay compared to higher fungi, but also that the hydrated cell wall may be advantageous for the enzymatic systems involved. Very little research has been conducted on the cellulolytic and hemicellulolytic systems on true soft rot fungi and few studies on the types of enzymes involved. Screening studies indicate, however, the effective endoglucanase activities, which is consistent with the alignment of hyphae with the cellulose microfibrils in wood cell walls with cavity formation. Soft rot fungi producing cavities and erosion in preservative-treated wood cell walls also have an effective system for immobilization of heavy metals in addition to causing decay.

For an overview of carbohydrate-degrading enzymes produced by wood decay fungi, readers should consult the CAZy database (www.cazy.org) and for fungal oxidoreductases (i.e., lignin-degrading enzymes) the FOLy (Fungal Oxidative Lignin Enzymes) database (https://foly-db.esil.univ-mrs.fr/) (Levasseur et al. 2008) and the more recently updated CAZy database that includes auxiliary activities and covers redox enzymes (http://www.cazy.org/Auxiliary-Activities.html). The aim of the databases is to provide an overview of cellulose- and lignin-degrading enzymes for biotechnical applications.

15.8 *Chaetomium* and Egyptian Papyrus

In 1973 Kowalik and Sadurska studied the microbiota of papyrus from samples of Cairo museums. Different fungi imperfecti, ascomycetes, and actinomycetes were isolated from samples of papyrus of Cairo museums. They recovered many fungi such as *Alternaria geophila, Botryodiplodia theobromae, Emericellopsis minima, Fusarium lactis, Helminthosporium sativum, Spondylocladium australe*, some species of the genus *Chaetomium*, and some actinobacteria related to genus *Streptomyces* which seemed to be specific for papyrus and/or for Egyptian climatic conditions. The genus *Chaetomium* and *Emericellopsis* may play a great role in decomposition of basic polymers of papyrus. Considering the nitrogen source of microbiota, it can be observed that papyrus-destroying microorganisms preferred ammonium to nitrate ions. It was found that papyrus-decomposing microorganisms may grow

equally well at 24–26 °C at 30 °C and some fungi are growing even at 42 °C. The members of the genus *Penicillium*, which prefer rather low temperatures and are frequent inhabitants of paper, were isolated from papyrus only once. By using the method of paper sheets damped with a 10 percent ethyl alcohol solution of thymol, pentachlorophenol, dichlorophene, and p-chloro-m-cresol as microbiocides, it could be concluded that only the last fungicide may assure the protection of papyrus. They recovered 12 species of *Chaetomium*; they were the following: *C. angustum, C. atrobrunneum, C. bostrychodes, C. cochliodes, C. elatum, C. fusiforme, C. globosum, C. indicum, C. ochraceum, C. olivaceum, C. trilaterale*, and *C. turgidopilosum.*

15.9 *Chaetomium* and Egyptian Archeological Wood

In 2019 Abdel-Azeem et al. studied the assessment of biodegradation in ancient archaeological wood from the Middle Cemetery at Abydos, Egypt. Abydos is a large, complex archaeological site located approximately 500 km south of Cairo in Upper Egypt. The site has served as a cemetery for thousands of years and is where most of the Early Dynastic royal tombs are located. North Abydos includes the Middle Cemetery and the North Cemetery, which are separated from each other by a wadi. The Middle Cemetery was the burial ground for important Sixth Dynasty (2407–2260 BC) officials and over time for thousands of elite and non-elite individuals as well. Excavations at the core area of the Old Kingdom mortuary landscape have revealed many culturally important wooden objects but these are often found with extensive deterioration that can compromise their preservation. The objectives of this study were to characterize the biodegradation that has taken place in excavated wooden objects, elucidate the type of wood degradation present, obtain information on soil properties at the site, and identify fungi currently associated with the wood and soils. Light and scanning electron microscopy studies were used to observe the micromorphological characteristics of the wood, and culturing on different media was done to isolate fungi. Identification of the fungi was done by examining morphological characteristics and extracting rDNA from pure cultures and sequencing the ITS region. Wooden objects, made from *Cedrus*, *Juniperus* and *Acacia* as well as several unidentified hardwoods, were found with extensive degradation and were exceedingly fragile. Termite damage was evident and frass from the subterranean termites along with sand particles were present in most woods. Evidence of soft rot attack was found in sections of wood that remained. Fungi isolated from wood and soils were identified as species of *Aspergillus*, *Chaetomium*, *Cladosporium*, *Fusarium*, *Penicillium*, *Stemphylium, Talaromyces,* and *Trichoderma*. Results provide important information on the current condition of the wood and gives insights to the identity of the fungi in wood and soils at the site. These results provide needed information to help develop conservation plans to preserve these degraded and fragile wooden objects.

Light microscopy (Fig. 15.9a) of wood sections revealed cavities within the secondary wall of tracheids that was characteristic of Type I soft rot. Advanced stages

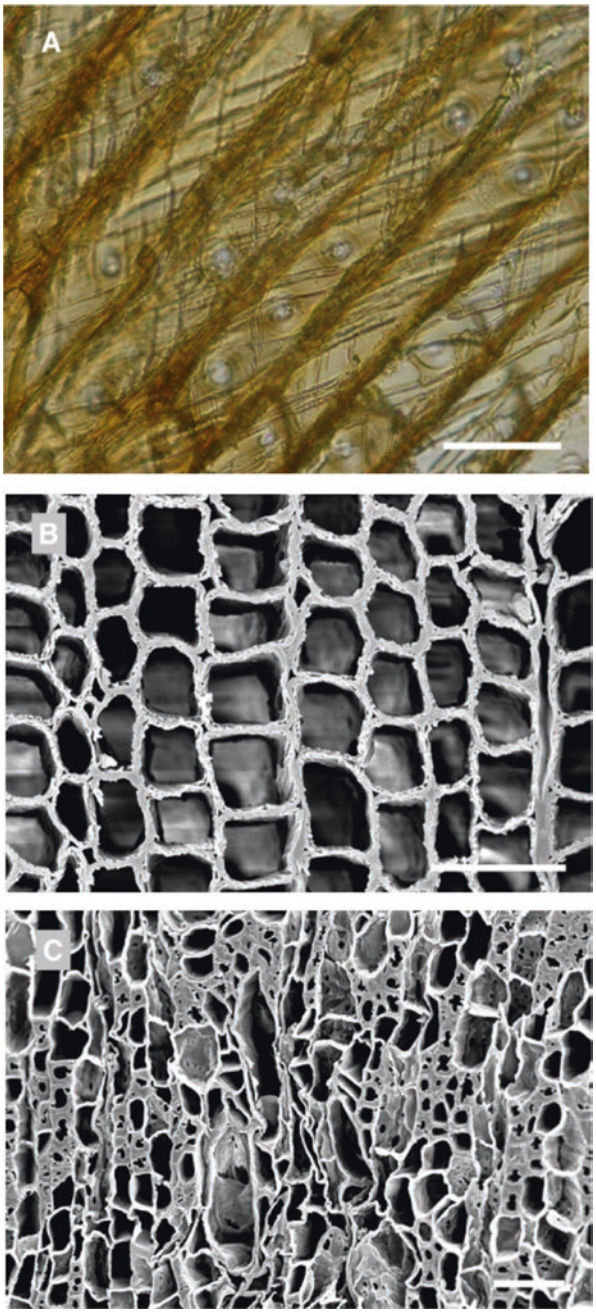

Fig. 15.9 showed the micromorphology of decayed wood. Tangential section (**a**) and scanning electron micrographs of transverse sections (**b** and **c**) from wooden objects with wood degradation. **a**) Type I soft rot in wood cells of Cedrus showing cavities that form inside the tracheid secondary walls. Soft rot cavities spiral within the cells. **b**) Small cavities are evident within the cell walls of the tracheids. **c**) An unknown hardwood with Type I soft rot and cavities within the secondary cell walls and also Type II soft rot causing an erosion of the entire secondary wall. Only a fragile middle lamella remains in some cells. Weakened cells appear distorted and some have collapsed. Scale bar = 50 μm (Abdel-Azeem et al. 2019)

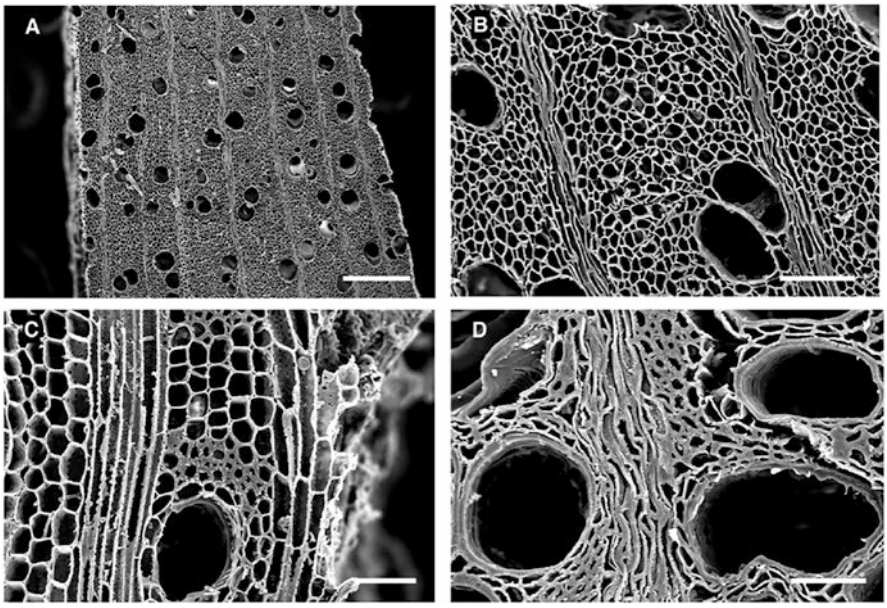

Fig. 15.10 showing scanning electron micrographs of transverse sections of degraded wood. **a** and **b**) Cell walls degraded by Type II soft rot from AMC 2013 field coffin sample 7. Secondary cell walls are degraded with most cells having only the middle lamella remaining. Although the wood structure can still be seen, the thin cell walls remaining are weak and fragile. **c**) Wood from coffin (AMC2013, Unit 28, Feat. 14, Burial 26.4) showing cells with Type II soft rot with degraded secondary walls and a thin middle lamella remaining as well as some cells with less severe attack that have some of the fiber secondary walls still present. **d**) Wood section from a box found in the Weni tomb (AMC01 level 1). The degraded cell walls caused by the soft rot have little strength and often break or collapse as seen in this micrograph. Scale bar in A = 250 μ and B, C, and D = 50 μ (Abdel-Azeem et al. 2019)

of decay were present and many soft rot cavities were seen in a spiral pattern within the secondary walls. In transverse sections, small cavities were seen within tracheids (Fig. 15.9a). Wood from objects that had been made of various hardwoods also had evidence of soft rot. Some cavities within secondary walls were present but more commonly observed was a Type II soft rot where the secondary cell walls were eroded (Figs. 15.9 and 15.10). In some cells with advanced decay, the entire secondary cell wall was removed leaving only the middle lamella.

The altered cells were often collapsed and distorted. Sections from other wooden objects showed similar patterns of degradation with Type II soft rot in objects made from hardwood (Fig. 15.9). The secondary walls were degraded leaving only a weak and fragile framework of the middle lamella and cell walls were often fractured and collapsed (Fig. 15.10).

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