INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 5, Issue 3, July-September 2014: 295-308 www.ijcs.uaic.ro

SURVEY FOR FUNGAL DECAYING ARCHAEOLOGICAL WOOD AND THEIR ENZYMATIC ACTIVITY

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Abstract

Fungi play a considerable role in deterioration/degradation of cultural heritage due to their enormous enzymatic activity. A total of 112 fungal isolates were identificated from selected archaeological wood objects located at different areas (Islamic Art Museum, Storage area of Cheops's Solar Boat, Excavation of Saqqara and Grand Egyptian Museum). Aspergillus spp. were predominant in all investigated samples. Thirty seven fungal isolates were screened for cellulases, pectinases and ligninases activity. Aspergillus brasiliensis Varga, Frisvad et Janos *and Penicillium duclauxii* Delacroix *exhibited high cellulolytic activity while Aspergillus amstelodami* (Mangin) Thom and Cruch *and Aspergillus parasiticus Speare have high pectinolytic activity. Seven fungal species showed ligninolytic potential activity based on their ability to oxidize dyes.*

Keywords: *Biodeterioration/biodegradation; Archaeological wood; Cultural heritage; Fungal cellulases; Pectinases; Ligninases.*

Introduction

The fungal colonization of art pieces presented in display rooms of museums, galleries or stored in depots is nowadays a significant problem for cultural heritage conservators [1]. Materials colonized by fungi usually undergo changes in their chemical and physical characteristics [2], leading to biodeterioration/biodegradation and must not be neglected due to the increasing aesthetic value of art objects as well as the impact on health of the curator.

Moisture is a key factor to achieving the microbial decay. Even in buried tombs from arid regions some moisture is usually present, allowing selected microbes to grow and progressively degrade the wood. In addition to moisture, many other factors influence microorganism growth, such as temperature, pH, nitrogen, and other nutrients [3]. Favorable conditions allow rapid colonization by fungi and fast decomposition, but unfavorable conditions

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usually cause develop of other organisms which tolerate the extreme environmental conditions to dominate. The type of wood and presence of extractives within the wood cells also influences decay [4].

The fungus represent the principal factors of wood degradation in terrestrial sites and decay can be classified into broad categories of white, brown, and soft rot depending on the color and texture of the residual wood after decay [3, 5]. In historical and cultural wooden objects soft rot decay has been described in wood samples from a range of sites and environments, for example waterlogged archaeological wood, construction timbers in great houses lived-in by native Americans, ancient wooden coffins and tomb structures, a wide range of ancient wooden Egyptian objects, and the historic expedition huts in Antarctica [3, 6 -12]. This type of decay occurs generally when wood is exposed to excessive moisture, but it can also occur in dry environments [7]. Before wood degradation occurs by the actions of extracellular enzymes and other metabolites produced by fungi, the type and sequence of attack on wood cell wall components (i.e. cellulose, hemicelluloses and lignin) can vary depending on the specific degradative actions of the fungus. However fungi are well known as agents of decomposition of organic matter, in general, and of cellulosic substrate in particular [13]. Pectinases are the first cell wall degrading enzymes that are secreted by pathogens and are important virulence factors [14]. Pectinases are group of enzymes that attack pectin and depolymerize it by hydrolysis and trans-elimination as well as by de-esterification reactions, which hydrolyses the ester bond between carboxyl and methyl groups of pectin [15]. They weaken the plant cell wall and expose other polymers to degradation by hemicellulases and cellulases. Enzymes hydrolyzing cellulose have been categorized as endoglucanases and exoglucanases that act synergistically [16].

By contrast, lignin is highly resistant in chemical and biological degradation, and confers mechanical resistance to wood. The main groups of fungal ligninolytic enzymes (ligninases) are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac). Although many different types of microorganisms are capable to degrade using cellulases as carbon and energy sources, a much smaller group of microorganisms especially fungi, have evolved the ability to break down lignin. Different group of fungi have been reported as producers of ligninolytic enzymes [17]. Most of the lignocellulolytic fungi secrete extracellular enzymes released in the presence or absence of inducers in the media [18, 19].

Materials and methods

Samples collection

Various biodeteriorated/biodegradated wood samples were collected from different archaeological objects in different areas (Islamic Art Museum, Storage area of Cheops's Solar Boat, Excavation of Saqqara and Grand Egyptian Museum) (Table 1).

Isolation and Identification of fungi

Isolation of fungi was carried out using sterile cotton swab technique or biodeteriorated/biodegradated wood pieces technique. In biodeteriorated/biodegradated wood pieces technique, very small biodeteriorated/biodegradated wood separated from the original ancient wood objects were surface sterilized by soaking for one minute in a 5% hypochlorite solution, followed by two rinses in sterile distilled water, sliced and cultured on a variety of enriched and semi-selective media prepared as agar plates.

The used media were potato dextrose agar, PDA (Difco), cellulose agar medium and Lignin Cellulose medium (LCM) [20], supplemented with chloramphenicol (100mg/L) to prevent bacterial contamination. Isolated fungi were identified at least to the genus level

depending on their morphological characteristics on different culture media (Czapek agar media- Malt extract agar media) using Upright light microscopes (Zeiss) with the aid of the following references: [21-24] and identification of some species were confirmed by Assiut University Mycological Center (AUMC), Egypt.

Table 1. Types and sites of collected samples

Current Location	Name of object	Period/ Dynasty	Number	Code of sample
Islamic Art Museum	Stucco window surrounded by wooden frames	Osmanic period	No. 9393/1	Code.1
Excavation of Saqqara	Wooden mask from coffin carrying the remains of the plaster layer	Late period	No.341wl/2012	Code.2
	2 Part of wooden coffin and contains drawings of four God of the dead (Anubis and Horas)	Late period	(No. 330wl/2012)	Code.3
	Cover of wooden coffin loses both of the chest and face. This part contains the drawings represent died and canopic containers. There are also drawings of some of the dead gods.	Late period	No. 340wl/2012)	Code.4
Grand Egyptian Museum	Abo-Rawash boat	Archaic Period -First dynasty period- king Den	-	Code.5
	3 Offering food box of Tut Ankhamun	Modern period- Eighteenth dynasty	Gem 6827, 6831 and 6842	Code. 6,7 and 8 respectively
	3 Wooden black box for shawabti statute of Tut Ankhamun	Modern period- Eighteenth dynasty	Gem 478 , 476 and 477	Code. 9,10 and11 respectively
	2 model of boat of Tut Ankhamun	Modern period- Eighteenth dynasty	Gem 4621 and 4618	Code.12 and13 respectively
Storage area of Cheops's Solar Boat	Falling parts of boat have been replaced with other parts during restoration	Old kingdom - Fourth dynasty	-	Code.14

Screening for cellulolytic activity

Thirty seven fungal isolates were preliminary screened for cellulolytic enzyme activity using disc plate method. The basal medium described by S. Bland and E.E. Douglas [25] was used for cellulose production. The developed colonies were further incubated at 50°C for 8 hour to detect the clear zone around their growth [26]. The diameter of the clear zone formed around the colonies corresponds to the enzymatic activity of the culture and was determined using Rosebengal as described by S. Bland and E.E. Douglas [25].

Quantitative assay for cellulase activity. Filter paper assay

Cellulase activity was determined by a method of M. Mandels *et al.* [27]. An aliquot of 0.5mL of cell-free culture supernatant was transferred to a clean test tube and 1.0mL of sodium acetate buffer (pH = 4) was added. Whatman #1 filter paper strip (6cm ×1.0cm) was added to each tube. The mixture was incubated in a water bath at 50°C for 30min [28, 29] followed by an addition of 1ml of 3,5 dinitrosalicylic acid reagent [30]. Tubes were then placed in a boiling water bath for 5min and then in an ice-bath. The developed colour was detected at wavelength 540nm using T 60 U Spectrophotometer. The amount of reducing sugar liberated was quantified

using glucose as standard. One unit of cellulase is defined as the amount of enzyme that liberates 1μmol of glucose equivalents per minute under the assay conditions [31].

Carboxymethyl Cellulase "CMCase" (endoglucanase) assay

CMCase activity was determined by the method of M. Mandels *et al.* [27] as described above with a modification of using 0.5mL of 1% (w/v) CMC as a substrate instead of filter paper strips. Enzyme activity was determined in terms of International Unit (IU) which is defined as an amount of enzyme that produces 1 μ mole of glucose per minute.

Pectinase assay

Pectinase activity was determined using citrus pectin as substrate. The reaction mixture, containing equal amounts of 1% pectin prepared in sodium acetate buffer (0.1M; pH = 5.0) and suitably diluted crude enzyme, was incubated at 50°C in water bath for 30 minutes. The reaction was stopped with 1.0mL dinitrosalicylic acid solution [30] and then the mixture was boiled for 10min and cooled. The colour was detected at wavelength 540nm. The amount of reducing sugar released was quantified using galacturonic acid as standard.

Soluble protein assay

Protein content of the culture supernatant was determined according to the method described O.H. Lowry *et al*. [32]. One ml of the crude enzyme supernatant was used and 5.0mL reaction mixture was added in a clean dry test tube. The tubes were kept at room temperature for 10 minutes. Then 0.5mL of Folin reagent (Fluka) was added to the previous mixture. After 30 minutes, the optical density of the sample was determined spectrophotometry at 700nm against blank.

Screening for ligninolytic enzyme on solid medium with isolated fungi

Synthetic phenolic reagents such as 0.01% guaiacol was used as an indicator for the ligninolytic enzyme reaction with fungal isolates on 2% MEA or PDA plates [33].

Statistical analysis

Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncuna) using the Statistical Package for the Social Science (SPSS) version 17. Duncan's multiple range tests were used at significance P = 0.05 according to F. Colao *et al.* [34].

Results and Discussion

Isolation and identification of fungi

A total number of 112 fungal isolates were selected from different wooden artifacts located at different area and then identified. The isolates belong to the phyla *Deuteromycota* (10 genera), *Ascomycota* (4) and *Zygomycota* (1). 95 isolates were from plates incubated at 28°C and 17 from plates incubated at 37°C. The number of fungi isolated on each medium was as follows: 61 isolates on PDA; 23 isolates on Cellulose agar Medium and 28 isolates on Lignin Cellulose medium (LCM) (Table 2). All fungal isolates were transferred by sub-culturing from hyphal tips, colonies or spores to fresh PDA plates for pure culture.

Biodeteriogens/biodegradogens are organisms involved in deterioration and degradation of artifacts. They are very specific for each type of artifact in accordance with its chemical structure and environment. They also have different nutritional requirements and act directly or indirectly on the substrate. The main intrinsic reasons for the permanent establishment of microorganisms on ancient surfaces are their capacity of adhesion, oligotrophy, metabolic flexibility and tolerance to adverse conditions. The adhesion of microorganisms to a substrate is the result of cell hydrophobicity and of excreted polymeric substance contained by sheaths, capsules and slimes [35].

Table 2. Fungi isolated from different archaeological objects in different locations (Islamic Art Museum, Storage area of Cheops's Solar Boat, Excavation of Saqqara and Grand Egyptian Museum).

Fungal isolate	Archaeological objects									Frequency %	Incubation temperature		Isolation media		
	1	2	3	4	5	6 -11	12	13	14		28°C	37°C	PDA	Cellulose agar media	LCM
Alternaria alternate	-	-	-	-	+	-	-	-	-	7.1%	+	-	+	-	+
Alternaria sp.	-	-	-	-	-	-	-	-	+	7.1%	+	-	+	-	-
Aspergillus aegyptiacus (AUMC No. 9488)	-	-	-	+	-	-	-	-	-	7.1%	+	-	-	+	-
A. amstelodami (AUMC No. 9486)	-	-	-	+	-	-	-	-	-	7.1%	+	-	-	-	+
A. brasiliensis (AUMC No. 9482)	+	-	+	+	+	-	-	+	-	35.7%	+	+	+	+	+
A. flavipes	+	-	-	-	-	-	-	-	-	7.1%	+	NT	+	-	-
A. flavus group	-	-	-	-	-	-	-	-	+	7.1%	-	+	+	-	-
A. flavus var *columnaris*	+	-	-	+	-	-	-	+	-	21.4%	+	-	+	+	+
A. niger	-	-	-	-	-	-	-	-	+	7.1%	-	+	+	-	+
A. ochraceus	-	-	+	-	-	-	-	-	-	7.1%	+	-	+	+	-
A. parasiticus (AUMC No. 9483)	-	-	+	-	-	-	-	-	+	14.2%	+	+	+	+	+
A. terreus	-	-	-	+	-	-	-	-	+	14.2%	+	-	+	-	+
A. terreus Var. *africanus* (AUMC No. 9485)	-	+	-	-	-	-	-	-	-	7.1%	+	-	+	-	-
A. versicolor group(1)	-	+	-	-	-	-	-	-	-	7.1%	+	+	-	-	+
A. versicolor group(2)	-	-	-	+	+	-	-	-	-	14.2%	+	-	+	+	-
Aspergillus sp. (1)	+	-	-	-	-	-	-	-	-	7.1%	+	NT	+	-	-
Aspergillus sp.(2)	-	-	-	-	-	-	-	-	+	7.1%	+	-	-	+	-
Chaetomium sp.	-	-	-	-	-	-	-	-	+	7.1%	+	-	+	-	-
Cladosporium cladosporioides	-	-	-	-	+	-	-	-	-	7.1%	+	-	+	+	+
Cladosporium sp.(1)	+	-	-	+	-	-	-	-	-	14.2%	+	-	+	+	-
Cladosporium sp.(2)	-	-	-	-	-	-	-	-	+	7.1%	+	-	+	+	+
Emericella nidulans	-	-	-	+	-	-	-	-	-	7.1%	-	+	-	-	+
Epicoccum nigrum (AUMC No. 9489)	+	-	-	-	-	-	-	-	-	7.1%	+	NT	+	-	+
Eurotium amstelodami	-	+	-	-	-	-	-	-	-	7.1%	+	-	+	-	-
E. chevalieri	-	+	-	-	-	-	-	-	-	7.1%	+	-	+	-	-
E. glaucus	+	-	-	-	-	-	-	-	-	7.1%	+	NT	+	-	+
Paecilomyces sp.	-	-	-	+	-	-	-	-	-	7.1%	+	-	+	-	-
Pencillium capsulatum (AUMC No. 9491)	-	-	-	+	-	-	-	-	-	7.1%	+	-	+	-	-
P. duclauxii (AUMC No. 9487)	-	-	-	+	-	-	-	-	-	7.1%	-	+	-	-	+
Penicillium sp. (1)	-	-	-	+	-	-	-	-	-	7.1%	+	-	+	-	+
Penicillium sp. (2)	+	-	-	-	+	-	-	-	+	21.4%	+	-	+	+	+
Penicillium sp. (3)	-	-	+	-	-	-	+	-	-	14.2%	+	+	+	+	-
Penicillium sp. (4)	-	-	+	-	-	-	-	-	-	7.1%	+	-	-	+	-
Phoma sp.(AUMC No. 9481)	-	-	-	-	-	-	-	-	+	7.1%	+	-	+	-	-
Scopulariopsis sphaerospora (AUMC No. 9490)	-	+	-	-	-	-	-	-	-	7.1%	+	-	-	-	+
Stemphylium botryosum (AUMC No. 9484)	-	-	-	-	+	-	-	-	-	7.1%	+	-	+	-	-
Syncephalastrum racemosum	+	-	-	-	-	-	-	-	-	7.1%	+	NT	+	-	-
Trichophyton sp. (1)	-	-	-	-	+	-	-	-	-	7.1%	+	-	-	+	-
Trichophyton sp. (2)	-	-	-	-	+	-	-	-	-	7.1%	+	+	+	+	-
Ulocladium sp.	-	-	-	-	+	-	-	-	-	7.1%	+	-	+	-	-
Total No. of isolates						112					95	17	61	23	28

Note + = presence of fungal isolates; - = absence of fungal isolates & NT means not tested.
Object 1= wooden frames of Stucco window; object 2= wooden mask No.341WL/2012; object 3= Part of wooden coffin No.330WL/2012; object 4= Cover of wooden coffin No. 340WL/2012; object 5= Abo-Rawash boat; object 6= Offering food box of Tut Ankhamun Gem 6827; object 7= Offering food box Gem 6831; object 8= Offering food box Gem 6842; object 9= Wooden black box for shawabti statute Gem 478; object 10= Wooden black box for shawabti statute Gem 476; object 11= Wooden black box for shawabti statute Gem 476; object 12= model of boat Gem 4621; object 13= model of boat Gem 4618 and object 14 = Falling wooden parts of Cheops's Solar boat.

The obtained results showed that 26 isolates, represented 10 fungal species were obtained from Storage area of Cheops's Solar Boat. The most dominant fungi on falling wooden parts of Cheops's Solar boat (object 14) were *Aspergillus* (5 spp.), *Penicillium* (1sp.), *Alternaria* (1 sp.), *Cladosporium* (1 sp.), *Chaetomium* (1 sp.), and *Phoma* (1 sp.). The results also, showed that 24 isolates, representing 9 fungal species were obtained from Islamic Art Museum. The most dominant fungi on wooden frames of Stucco window No. 9393/1 (object 1) were *Aspergillus* (4 spp.), *Penicillium* (1sp.), *Cladosporium* (1 sp.), *Syncephalastrum* (1 sp.), *Epicoccum* (1 sp.), and *Eurotium* (1 sp.). While the examination of Abo-Rawash boat (object 5) from Grand Egyptian Museum revealed the presence of 22 isolates, representing 9 fungal species were *Aspergillus* (2 spp.), *Trichophyton* (2 spp.), *Penicillium* (1sp.), *Cladosporium* (1 sp.), *Alternaria* (1 sp.), *Ulocladium* (1 sp.), and *Stemphylium* (1 sp.). Also, 16 isolates

representing 12 fungal species were obtained from Excavation of Saqqara. The most dominant fungi on Cover of wooden coffin No. 340WL/2012 (object 4) were *Aspergillus* (6 spp.), *Penicillium* (3 spp.), *Cladosporium* (1 sp.), *Emericella* (1 sp.), and *Paecilomyces* (1 sp.). On the other hand, the examination of object 3 (Part of wooden coffin No. 330WL/2012) revealed the presence of 11 isolates, representing 5 fungal species from genera *Aspergillus* (3 spp.), and *Penicillium* (2 spp.). Also object 2 (wooden mask No. 341WL/2012) examination revealed the presence of 6 isolates, representing 5 fungal species from genera *Aspergillus* (2 spp.), *Eurotium* (2 spp.) and *Scopulariopsis* (1 sp.). The results also showed that 5 isolates, representing 2 species of *Aspergillus* were obtained from boat model of Tutankhamun Gem No. 4618 (object 13) and one species of *Pencillium* from boat model Gem No. 4621 (object 12). The results of objects 6 to 11 showed no fungal colonization on the used media due to the controlled condition system.

These results are in line with those obtained by A.M. Abed ElHameed [36] who isolated *Alternaria alternata*, *Aspergillus flavus*, *A. versicolor*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *Mortierella* sp., and *Pencillium chrysogenum* from wooden coffins in Egyptian museum. Also, similar results were obtained by H. Hanna [37] who isolated species of the genera *Alternaria*, *Aspergillus*, *Pencillium, Rhizopus* and *Stemphylium* from wood specimens of Cheops boat. G.M. Ljaljevic *et al.* [38] isolated species of *Absidia*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Neurospora*, *Penicillium*, *Rhizopus*, *Syncephalastrum* and *Trichoderma* from the wooden substratum in the quarantine room of the Cultural Center of Belgrade. Also, D. Bridžiuvienė and V. Raudonienė [39] isolated *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, *Phoma*, *Trichoderma* and *Ulocladium* genera from treated wood. Also, S.M.N. Maghazy *et al.* [40] isolated 106 fungal species related to 41 genera collected from 32 places (16 Pharaonic, 8 Greek Roman and 8 Coptic) in Minia governorate. From the isolated genera, *Alternaria, Aspergillus, Penicillium, Cladosporium, Chaetomium, Emericela, Epicoccum, Paecilomyces, Phoma*, *Stemphylium*, *Syncephalastrum,* and *Ulocladium*.

The most common fungi isolated from different wooden objects belonged to the following genera *Aspergillus* (35.78%), followed by *Cladosporium* (28.34%) and *Pencillium* (18.33%), while other fungal species were detected at low incidence rate (Table 3).

H.A. Burge *et al*. [41] reported that the most prevalent molds growing in indoor environments were *Penicillium*, *Aspergillus*, and *Cladosporium* species. *Penicillium* and *Aspergillus* spp. can produce numerous mitospores, or conidia, which are easily dispersed by the air, where they include species that can grow at relatively lower moisture availability than other cellulolytic fungi. These fungi and certain species of *Alternaria* are usually as contaminants or biodeterioration agents in many different habitats and materials, including those considered as representative of historical and cultural heritage [42]. *Penicillium* usually forms green colonies on wood surface nevertheless they rarely have been reported to cause soft rot [43]. *Cladosporium* sp. is cosmopolitan and has a high abundance in air samples in many areas of the world. *Cladosporium cladosporioides* isolated from freshly sawn wood [44] and Chromated copper arsenate (CCA)-treated wood [43], and can degrade historic wood [45]. Furthermore, this species has been found causing soft-rot type 2 attack on birch wood [46]. *Cladosporium*, *Phoma* as well as *Ulocladium* have black pigment (melanin) that caused distinctly seen dark grey discoloration on wood joints studied [22]. J.-J. Kim *et al*. [43] reported that even 45% of the isolates from CCA-treated wood radiata pine board stored at the yard in Korea have been *Phoma* species though in the study of D. Bridžiuvienė and V. Raudonienė [39] it was isolated only once as in the present study. The genus *Chaetomium* includes more than 80 different species and many of them have been reported to occur on or in wood [47]. T. Nilsson [48] demonstrated that *C. globosum, C. elatum,* and *C. funicola*, can cause weight losses of 20% or more on birch wood, and all three species can produce both type 1 and type 2 soft-rot attack, and *Chaetomium* thermophilum can also cause soft-rot attack on wood, with weight losses of 13.8%, at very high temperature. However, for this case study, the

deteriogenic potential of the *Chaetomium* sp. isolated cannot be stated, as they were not identified to the species level.

Table 3. Occurrence of different fungi isolated from different wooden objects

No. of Isolates	Fungal isolate	Occurrence %	Phylum
1	*Alternaria alternata*	2.68%	Deuteromycota
2	*Alternaria* sp.	0.89%	
3	*Aspergillus aegyptiacus*	0.89%	
4	*A. amstelodami*	0.89%	
5	*A. brasiliensis*	11.61%	
6	*A. flavipes*	0.89%	
7	*A. flavus* group	1.79%	
8	*A. flavus* var *columnaris*	8.03%	
9	*A. niger*	3.57%	
10	*A. ochraceus*	1.79%	
11	*A. parasiticus*	9.82%	
12	*A. terreus*	1.79%	
13	*A. terreus* Var. *africanus*	0.89%	
14	*A. versicolor* group(1)	1.79%	
15	*A. versicolor* group (2)	2.68%	
16	*Aspergillus* sp. (1)	0.89%	
17	*Aspergillus* sp.(2)	0.89%	
18	*Chaetomium* sp.	0.89%	Ascomycota
19	*Cladosporium cladosporioides*	6.25%	Deuteromycota
20	*Cladosporium* sp.(1)	2.68%	
21	*Cladosporium* sp.(2)	5.36%	
22	*Emericella nidulans*	0.89%	Ascomycota
23	*Epicoccum nigrum*	0.89%	Deuteromycota
24	*Eurotium amstelodami*	0.89%	Ascomycota
25	*E. chevalieri*	0.89%	
26	*E. glaucus*	1.79%	
27	*Paecilomyces* sp.	0.89%	Deuteromycota
28	*Pencillium capsulatum*	0.89%	
29	*Penicillium* duclauxii	0.89%	
30	*Penicillium* sp.(1)	0.89%	
31	*Penicillium* sp.(2)	7.14%	
32	*Penicillium* sp.(3)	4.46%	
33	*Penicillium* sp.(4)	0.89%	
34	*Phoma* sp.	0.89%	
35	*Scopulariopsis sphaerospora*	0.89%	
36	*Stemphylium botryosum*	0.89%	
37	*Syncephalastrum racemosum*	1.79%	Zygomycota
38	*Trichophyton* sp. (1)	0.89%	Ascomycota
39	*Trichophyton* sp. (2)	2.68%	
40	*Ulocladium* sp.	1.79%	Deuteromycota

Screening for fungal Cellulases

37 fungal isolates obtained were tested for cellulases production [FPase and endoglucanase (CMCase)]. During the present study, filter paper assay for cellulase activity was found to be highest in *Aspergillus brasiliensis* with 24.48U/mL and endoglucanase with an activity level represented by 18.23U/mL, followed by *Penicillium duclauxii* which gave FPase (24.27U/mL) and CMCase (17.74U/mL). The activities ranged from 14.96 to 24.48U/mL for FPase. Not all the tested species were capable of exhibiting hydrolysis for CMC so the activities ranged from very low or no detectable to with the highest level (18.23U/mL) for endoglucanase assay. The two species *Aspergillus brasiliensis* and *Penicillium duclauxii* exhibited the highest extracellular cellulase activities compared to other isolates as shown in activity assay performed for all isolates (Table 4). Soluble protein also varied greatly as indicated in Table 4. There was

a negative correlation between cellulase activity (diameter of clear zone) and soluble protein (r = -0.08437).

Table 4. Total cellulases (FPase), endoglucanase (CMCase) produced by different fungal isolates

No.	Fungal isolate	Diameter of Clear zone (cm)	FPase (U mL-1)	CMCase (U mL-1)	Soluble protein (µg/ml)
1	*Alternaria alternata*	4.06 ± 0.05^{de}	19.42 ± 0.37^{de}	10.70 ± 0.30^{efg}	292
2	*Alternaria* sp.	3.20 ± 0.20^{kl}	17.67 ± 0.31^{ghi}	11.79 ± 0.43^{bcde}	296
3	*Aspergillus aegyptiacus*	3.53 ± 0.11^{ghijk}	18.23 ± 0.73^{fgh}	10.59 ± 0.64^{fg}	141
4	*A. amstelodami*	3.86 ± 0.15^{efg}	21.60 ± 0.41^{bc}	11.19± 0.55^{cdefg}	240
5	*A. brasiliensis*	5.80 ± 0.17^{a}	24.48 ± 0.52^{a}	18.56 ± 0.80^{a}	188
6	*A. flavipes*	3.76 ± 0.20^{efgh}	18.13 ± 0.28^{fgh}	10.59 ± 0.70^{fg}	195
7	*A. flavus* group	3.27 ±0.25^{jkl}	15.96 ± 0.49^{klmn}	ND	269
8	*A. flavus* var *columnaris*	3.2 0± 0.26^{kl}	16.15 ± 0.38^{klm}	11.49 ± 0.67^{bcdef}	229
9	*A. ochraceus*	4.06 ± 0.20^{de}	20.81 ± 0.74^{c}	10.90 ± 0.70^{cdefg}	202
10	*A. parasiticus*	4.53 ± 0.32^{c}	19.65 ± 0.53^{d}	11.49 ± 0.59^{bcdef}	227
11	*A.terreus*	4.30 ± 0.36^{cd}	21.30 ± 0.70^{bc}	12.42 ± 0.35^{b}	164
12	*A. terreus* var. *africanus*	3.23 ± 0.21^{jkl}	18.62 ± 0.54^{defg}	11.29 ± 0.87^{cdefg}	271
13	*A. versicolor* group(1)	2.03 ± 0.06^{o}	15.41 ± 0.81^{lmn}	11.89 ± 0.78^{bcd}	203
14	*A. versicolor* group (2)	2.43 ± 0.15^{n}	15.46 ± 0.50^{lmn}	10.33 ± 0.35^{g}	232
15	*Aspergillus* sp. (1)	3.06 ± 0.11^{lm}	16.74 ± 0.30^{ijk}	ND	221
16	*Aspergillus* sp.(2)	3.40 ± 0.17^{hijkl}	18.73 ± 0.88^{defg}	11.09 ± 0.80^{cdefg}	146
17	*Chaetomium* sp.	4.56 ± 0.23^{c}	22.00 ± 0.38^{b}	10.70 ± 0.55^{efg}	200
18	*Cladosporium cladosporioides*	3.43 ± 0.11^{hijkl}	18.33 ± 0.56^{efgh}	ND	177
19	*Emericella nidulans*	4.50 ± 0.17^{c}	21.11 ± 0.47^{bc}	10.99 ± 0.36^{cdefg}	224
20	*Epicoccum nigrum*	2.36 ± 0.05^{no}	15.36 ± 0.71^{lmn}	ND	222
21	*Eurotium amstelodami*	3.33 ± 0.15^{ijkl}	17.43 ± 0.54^{hij}	ND	277
22	*E. chevalieri*	3.95 ± 0.23^{ef}	19.22 ± 0.37^{def}	10.99 ± 0.79^{cdefg}	149
23	*E. glaucus*	3.33 ± 0.25^{ijkl}	16.84 ± 0.69^{ijk}	ND	275
24	*Paecilomyces* sp.	3.60 ± 0.17^{fghij}	18.73 ± 0.68^{defg}	10.80 ± 0.58^{defg}	136
25	*Pencillium capsulatum*	2.76 ± 0.15^{m}	15.85± 0.95^{klmn}	11.98 ± 0.63^{bc}	180
26	*P.duclauxii*	4.93 ± 0.21^{b}	24.27 ± 0.39^{a}	17.74 ± 0.40^{a}	255
27	*Penicillium* sp.(1)	3.66 ± 0.15^{fghi}	16.74 ± 0.65^{ijk}	ND	221
28	*Penicillium* sp.(2)	3.26 ± 0.30^{jkl}	16.35 ± 0.75^{jkl}	ND	193
29	*Penicillium* sp.(3)	3.40 ± 0.26^{hijkl}	16.84± 1.12^{ijk}	ND	258
30	*Penicillium* sp.(4)	2.36 ± 0.15^{no}	15.06 ± 0.67^{mn}	10.70 ±0.98^{efg}	173
31	*Phoma* sp.	3.43 ± 0.11^{hijkl}	18.03 ± 0.53^{gh}	ND	86
32	*Scopulariopsis sphaerospora*	2.03 ± 0.06^{o}	15.16 ± 0.42^{mn}	11.19 ± 0.66^{cdefg}	256
33	*Stemphylium botryosum*	3.53 ± 0.23^{ghijk}	17.74 ± 0.84^{ghi}	10.99 ± 0.64^{cdefg}	230
34	*Syncephalastrum racemosum*	2.83 ± 0.15^{m}	14.96 ± 0.49^{n}	11.49 ± 0.54^{bcdef}	258
35	*Trichophyton* sp. (1)	3.50 ± 00^{hijk}	18.02 ± 0.57^{gh}	11.59 ± 1.01^{bcdef}	205
36	*Trichophyton* sp. (2)	3.40 ± 0.17^{hijkl}	17.44 ± 0.78^{hij}	10.60 ± 0.69^{fg}	180
37	*Ulocladium* sp.	3.53 ± 0.15^{ghijk}	18.63 ± 0.55^{defg}	10.80 ± 0.32^{defg}	143

Note: ND= very low value (no detectable). Data are expressed as mean ± SD, Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's Multiple range test (P = 0.05). Correlation Coefficient (r) between Diameter of Clear zone and Soluble protein= -0.08437.

Aspergillus brasiliensis belongs to *Aspergillus* section *Nigri*. The filamentous fungus, *Aspergillus niger* secretes a complex array of degradative enzymes to hydrolyze cellulose efficiently and it is an important commercial source of cellulase [49]. Endo-β-1,4-glucanase is the main component of cellulose degradation by *A. niger* and has been classified as a member of the glycosyl hydrolase family 12 [50]. S. Nwodo-Chinedu *et al*. [51] isolated some cellulolytic fungi from wood-wastes in Lagos, Nigeria. The strains of *A. niger* (ANL 301) and *P. chrysogenum* (PCL501) isolated by the group were reported to produce cellulases [52] and

xylanases [53]. Thus, these fungi produce the full complement of enzymes required for the hydrolysis of cellulosic biomass.

Pectinolytic and ligninolytic activities

The isolated cultures were screened for their pectinolytic and ligninolytic activities. The polygalacturonase production ranged from 48.76 - 420.84U/mL in which *Aspergillus amstelodami* and *Aspergillus parasiticus* recorded the maximum polygalacturonase production (table 5). There was a positive correlation between Pectinase activity and soluble protein (r = 0.40232). Soluble protein varied greatly from cellulase activity as indicated in Table 4 about pectinase activity as in table 5 due to different media used with different nitrogen source.

Table 5. Pectinolytic activity of tested fungal isolates

No. of Isolates	Fungal isolate	Pectinase (U mL^{-1})	Soluble protein (μg /ml)
1	*Alternaria alternata*	131.04 ± 2.54^{l}	80
2	*Alternaria* sp.	48.76 ± 1.69^{x}	214
3	*Aspergillus aegyptiacus*	84.24 ± 1.78^{s}	257
4	*A. amstelodami*	420.84 ± 3.14^{a}	349
5	*A. brasiliensis*	213.71 ± 2.53^{d}	145
6	*A. flavipes*	58.67 ± 1.22^{w}	323
7	*A. flavus group*	72.34 ± 2.60^{tu}	312
8	*A. flavus* var *columnaris*	123.11 ± 2.35^{m}	364
9	*A. ochraceus*	185.16 ± 2.06^{f}	173
10	*A. parasiticus*	342.12 ± 2.87^{b}	1035
11	*A. terreus*	149.87 ± 1.21^{i}	241
12	*A. terreus Var. africanus*	168.91 ± 2.55^{g}	303
13	*A. versicolor* group (1)	104.46 ± 1.32^{o}	295
14	*A. versicolor* group (2*)*	89.99 ± 2.10^{r}	145
15	*Aspergillus* sp. (1)	90.99 ± 1.79^{r}	179
16	*Aspergillus* sp. (2)	75.32 ± 2.73^{t}	125
17	*Chaetomium* sp.	74.93 ± 3.04^{t}	286
18	*Cladosporium cladosporioides*	63.03 ± 1.72^{v}	116
19	*Emericella nidulans*	140.35 ± 2.32^{k}	154
20	*Epicoccum nigrum*	144.91 ± 2.92^{j}	89
21	*Eurotium amstelodami*	111.76 ± 3.53^{n}	254
22	*E. chevalieri*	133.61 ± 1.18^{l}	164
23	*E. glaucus*	141.15 ± 1.53^{jk}	190
24	*Paecilomyces* sp**.**	69.57 ± 2.38^{u}	227
25	*Pencillium capsulatum*	97.93 ± 2.15^{p}	339
26	*P. duclauxii*	202.21 ± 3.68^{e}	99
27	*Penicillium* sp.(1)	97.13 ± 2.55^{pq}	124
28	*Penicillium* sp.(2)	64.42 ± 1.56^{v}	167
29	*Penicillium* sp.(3)	85.24 ± 2.93^{s}	123
30	*Penicillium* sp.(4)	125.29 ± 1.90^{m}	102
31	*Phoma* sp.	164.74 ± 2.69^{h}	157
32	*Scopulariopsis sphaerospora*	93.56 ± 3.28^{qr}	222
33	*Stemphylium botryosum*	107.05 ± 2.80^{o}	430
34	*Syncephalastrum racemosum*	69.97 ± 2.62^{u}	103
35	*Trichophyton* sp. (1)	106.85 ± 1.88^{o}	631
36	*Trichophyton* sp. (2)	76.31 ± 3.22^{t}	140
37	*Ulocladium* sp.	230.37 ± 2.49^{c}	246

Data are expressed as mean ± SD , Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's Multiple range test (P = 0.05). Correlation Coefficient (r) between Pectinase and Soluble protein= 0.40232.

Aspergillus parasiticus belongs to *Aspergillus* section *Flavi*. S.S. Panda *et al*. [54] exhibited that *Aspergillus niger* and *A. flavus* are the highest potential for cellulase and pectinase enzyme activity, respectively. *Penicillium and Aspergillus* are among the most studied cellulolytic and pectinolytic fungi [55, 56].

Fungi developed on wood sometimes are able to degrade lignin as well. The phenoloxidases (peroxidase, tyrosinase and laccase) that take part in lignin decomposition are not characteristic of every fungus. The following isolates showed ligninase activity; *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Phoma* sp., *Chaetomium* sp., *Stemphylium*

botryosum, Trichophyton sp.(1), and *Trichophyton* sp. (2), while other ones showed no activities.

A.M. Mabrouk *et al*. [57] found that *Epicoccum purpurascens* gave laccase activity of 10.85 U/ml with specific activity of 59.30U/mg. Donnison *et al.* [58] found that *Phoma* sp. exhibited phenoloxidase activity where *Phoma* oxidize Mn(II) and deposit Mn oxides [59]. A. Verdin *et al*. [60] reported that the ability of *Cladosporium* strains to degrade polycyclic aromatic hydrocarbons. Also, D. Bridžiuvienė and V. Raudonienė [39] found that *Cladosporium cladosporioides* showed phenoloxidase activity. P.A. Geethanjali [61] showed that the phenol concentration of areca husk samples inoculated with *Chaetomium* sp. was high. According to C.K. Padmaja and D.L. Lavanya [62] Lignin rich coir pith, inoculated with *Chaetomium globosum* showed reduced lignin content greatly, because of the action of lignolytic enzymes of the fungi [63].

H. Bermek *et al*. [64] found that the fungus *Trichophyton rubrum* LSK-27, isolated from a decayed hardwood chip pile in the vicinity of Chonnam, Korea, produced high concentrations of MnP and low amounts of laccase so it degrades lignin mainly through MnP. This fungus was able to efficiently degrade lignin in both softwood and hardwood and secreted the lignin-degrading enzymes LiP, MnP and laccase during the degradation of wood [65]. So this fungus was classified as a white-rot fungus [64]. M.A. Lone *et al*. [66] found that the activity of the enzyme cellulase of *Trichophyton terrestre*, at a wide range of temperatures, exhibits higher activity at low temperature and thermal resistance even at 50°C.

The ability of the fungus to damage wood is dependent on many parameters prevailing at the site, such as synergism with the enzymes of other fungi [67, 68], antagonism with other organisms such as bacteria [69, 70], and environmental factors such as the moisture content [71], temperature [72], and pH [73, 74] of the soil.

Conclusion

Fungi play a very important role in deterioration of ancient wood antiques. In the present study, it could be concluded that the fungal cultures isolated from different wooden artifacts possess cellulolytic and pectinolytic activity. Among these fungal isolates, *Aspergillus brasiliensis* and *Penicillium duclauxii* were found to have maximum cellulase activity. *Aspergillus amstelodami* and *Aspergillus parasiticus* have high pectinolytic activity. The phenoloxidases (peroxidase, tyrosinase and laccase) that take part in lignin decomposition (and supposed to degrade preservatives of aromatic compound origin) were not characteristic of every fungus studied. Seven fungal species showed ligninolytic potential activity based on their ability to oxidize dyes.

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Received: December, 07, 2013
Accepted: July, 21, 2014