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Research article

The use of chitosan in protecting wooden artifacts from damage by mold fungi

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ABSTRACT

Background: Many buildings in Egypt e.g. museums, mosques and churches, do not possess controlled environments for minimizing the risks of damage of wooden artifacts due to the growth of fungi. Fungal damage usually appears as change in wood color, appearance of stains, and sometimes deformation of wooden surfaces. In this study we focused on the effect that some fungi exert on the properties of wooden artifacts and evaluated the effectiveness of different concentrations of chitosan on their protection against damage by mold fungi.

Results: Samples were collected from different monuments and environments, and fungi growing on them were isolated and identified. The isolated *Penicillium chrysogenum*, *Aspergillus flavus* and *Aspergillus niger* strains were used for the infestation of new pitch pine samples. The results revealed that the lightness of samples infected with any of the tested fungi decreased with increasing incubation times. XRD analysis showed that the crystallinity of incubated samples treated individually with the different concentrations of chitosan was lower than the crystallinity of infected samples. The crystallinity index measured by the first and the second method decreased after the first and second months but increased after the third and fourth months. This may due to the reducing of amorphous part by enzymes or acids produced by fungi in wooden samples.

Conclusions: The growth of fungi on the treated wood samples decreased with increasing the concentration of chitosan. Hence, it was demonstrated that chitosan prevented fungal growth, and its use could be recommended for the protection of archeological wooden artifacts.

1. Introduction

Wood consists of an orderly arrangement of cells with cell walls composed of varying amounts of cellulose, hemicelluloses, and lignin. The remaining components consist of various extracellular compounds [1]. When exposed to even moderate environmental conditions, wood deteriorates rapidly through a variety of biotic processes [2]. It is important to consider environmental factors such as moisture content, wood temperature, accessible nutrients, and the types of wood-decaying fungi present [3,4]. Fungi are widespread in nature and cause deterioration of wooden artifacts in a range of terrestrial and aquatic environments [5]. The structure of wood acts as a substrate template for fungal growth and development. During the damage and decay process, fungal hyphae may grow on the wood surfaces and into the wood via the xylem rays and then spread further. Depending on the type of decay, the fungal hyphae may be located in the cell lumen or even within all cell wall layers [3].

Most librarians, archivists, and museum personnel share the conviction that fungi should be eliminated. It is perhaps more appropriate and effective to concentrate on prevention. Here we restrict the meaning of the term fungicide to biocides in glacial acetic acid medium that can be applied directly to the surface of affected wood.

Chitosan has become a vital biocide and is used in different fields because of its high antimicrobial activity against various microorganisms [6]. Special interest has been shown in the use of chitosan for the control of wood degrading fungi. This may be due to

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the fact that chitosan toxicity exerts an effect on membrane permeability and on the architecture of the mycelium [7]. It was found that increasing concentrations of chitosan would cause an increase in chitin deposition, which would reflect changes occurring at morphological and ultrastructural level within the cell wall. Increase of the chitin content in the mycelium in the presence of increasing concentrations of chitosan suggesting that chitosan treatment enhanced deposition of cell wall [8]. It has been used widely due to its biodegradability, biocompatibility, antimicrobial effects, lack of toxicity, and anti-tumor properties [9]. Ahonkhai et al. [10] reported that chitosan is freely available, is cheap, and has antifungal and bacteriostatic properties. Chitin is naturally found in the exoskeleton of crustaceans and insects [11]. Chitosan occurs naturally in fungi of the order Mucorales. Commercial chitosan is mainly obtained by partially removing the acidic acid residues from the chitin of crustaceans. The polymer is characterized as chitosan according to the degree of deacetylation (DD), which is determined from the proportion of D-glucosamine and N-acetyl-D-glucosamine. Structurally, chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine. It is the most abundant basic polymer and is structurally similar to cellulose, which is composed of only one monomer (glucose). The solubility of chitosan depends on the amount of protonated amino groups in the polymeric chain, and therefore on the proportion of acetylated and non-acetylated D-glucosamine units. It is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric, and phosphoric [12,13,14,15,16,17,18,19]. In recent years, chitosan has been investigated as a chemical for protection of wood against fungal decay. Schmidt et al. [20] found that 2% chitosan treatment decreased the wood decay by two brown-rot fungi considerably. It has been used in aqueous solution, employing an impregnation technique [21].

Analyses and investigations are considered to be the most important tools for evaluation of the environment, and of materials that are used in conservation processes. The X-ray diffraction method has been used more and more extensively in the past twenty years for the characterization of different crystalline and non-crystalline materials of archeological, historical, and artistic interest [22]. Abdel-Maksoid and Al-Saad [23], Abdel-Maksoud [24], and Marutoiu et al. [25] have used X-ray diffraction for the determination of wood crystallinity. Bugheanu et al. [26], Picollo et al. [27], and Gelbrich et al. [28] have used FTIR for determination of the extent of deterioration of wood. Lo Monaco et al. [29] and Ozgenc et al. [30] have used UV spectrophotometry for measurement of color changes of wood treated with different applications.

In this study, we aimed to:

a) Isolate and identify mold fungi from monuments in different locations and environments of historical Cairo;
b) Study the effects of mold fungi that were isolated and identified on selected properties of pitch pine wood;
c) Evaluate the efficiency of different concentrations of chitosan for the protection of wood from mold fungi.

2. Materials and methods

2.1. Archeological samples

The fungi studied were isolated from the following sites:

a) The Mosque of Sabiile and Koutab Suleiman Agha Selehdar, which is located in El Muizz Street, Islamic Cairo. It was established in 1837–1839 AD (1253–1255 AH).
b) The Mosque of Abu Haribh, which was built by Prince Sayf al-Din Akjmas Ishaqi El Zahery between 1480 and 1481 AD (885–886 AH).
c) The Mosque of El Musafir Khana, situated at Darb Almsmt, El Gamaliya, and created by Mahmud Muharram. The first section was built in 1779 AD (880 AH), and the second in 1783 AD (884 AH).
d) The Mosque of El Mouayed Sheikh Al-Mahmoudi, Inside Bab Zuwayla, close to the El Soukary neighborhood. The construction of the mosque took about six years, from 1415 to 1421 AD (818–824 AH).

2.2. Isolation and identification of fungi

Isolation of fungi was done according to Abdel-Maksoud [24]. Pieces of deteriorated wood were placed into sterile plastic bags and were transferred to the laboratory, where their inner parts were exposed and sterile swabs were used to wipe along surfaces contaminated with fungi. The swabs were then gently wiped onto the surface of a potato-dextrose agar (PDA) medium in Petri dishes, and cultures were incubated at 28°C for 1–2 weeks. Individual fungal colonies were then recovered and transferred to Petri dishes with malt extract agar (MEA). Colonies from the established subcultures were used for the assessment of the morphological characteristics of the isolated fungi [31,32] and for their grouping into distinct species/morphotypes.

In addition, and for verifying initial taxonomic determinations based on morphological features, total genomic DNA of representative strains from each species/morphotype was extracted from mycelium. For the isolation of fungal DNA, 50 mL of potato-dextrose medium were inoculated by stock cultures, which were grown on MEA slants for 7 d at 27°C. Biomass was obtained after 2 d incubation at 27°C by centrifugation at 4000 × g for 10 min and washed twice with double distilled sterile water. Disruption of the cells was performed in a ceramic mortar using liquid nitrogen and a pestle until a white powder was obtained. Genomic DNA was extracted from 100 mg of biomass powder as instructed by the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St Louis, MO, USA). Elution was performed in two steps (100 μL each) and the presence of genomic DNA was realized by 1% agarose-gel electrophoresis.

PCR primers used by White et al. [33] design the amplification reactions for the ITS1-5.8S-ITS2 rDNA region were ITS1 (5′-TCCGTAGG TGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′), which were constructed by Eurofins Genomics (Ebersberg, Germany). The following PCR conditions were used for ITS amplification: the reaction was initiated at 95°C for 2 min followed by 35 cycles of 95°C for 20 s, 48°C for 30 s, 70°C for 15 s with a final extension step at 70°C for 2 min. PCR products were checked by agarose-gel electrophoresis using 1 kb DNA Ladder RTU (NIPPON Genetics EUROPE, Düren, Germany). The PCR products were directly sequenced by Eurofins Genomics (Ebersberg, Germany). All fragments were read in both directions and nucleotide sequences were submitted to GenBank database and their accession numbers are the following: KU243044, KU243045, KU243046 and KU243047.

Alignment of sequences was carried out through the use of the Clustal Omega software (http://www.clustal.org/omega/). Phylogenetic relationships were inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [34]. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated by the Maximum Composite Likelihood (MCL) approach. The branch swap filter was set at 'strong', and all sites were used for the analysis including gaps. Bootstrap values were derived from a total of 10,000 replicates. Pertinent analyses were conducted through the use of MEGA 6 software [35]. Eleven additional sequences from related (to this study's material) fungal taxa were retrieved from GenBank, and were included in the phylogenetic analysis. Among them, *Mucor fragilis* was used as outgroup.

2.3. Fungal strains

In this study, the three active strains *Aspergillus niger, Aspergillus flavus, and Penicillium chrysogenum* were isolated from wood samples taken from the different locations mentioned above.