

DOI: 10.5281/zenodo.3956802

ISOLATION AND CHARACTERIZATION OF MICROBIAL INFECTION OF POLYCHROME WOODEN COFFIN FROM THE 26TH DYNASTY, EGYPT

Abdelmoniem M. Abdelmoniem*¹, Naglaa Mahmoud¹, Wael S. Mohamed²

¹Conservation Department, Faculty of Archaeology, Fayoum University, Fayoum, Egypt

²Polymer Department, National Research Centre, Dokki, Giza, Egypt

Received: 01/06/2020

Accepted: 24/07/2020

Corresponding author: Abdelmoniem M. Abdelmoniem (ama63@fayoum.edu.eg)

ABSTRACT

The present study focuses on the definition of fungi and bacteria from the late period wooden coffin at Saqqara dating back to the 26th dynasty (664–525 BC). It highlights the importance of the black resin layer in resisting microbial infection. This polychrome wooden coffin was covered by a ground layer and a painted layer externally and by a layer of black resin internally. The wooden coffin had a lot of deterioration factors, e.g. the loss of the painted layer and ground layers, as well as a thick layer of dust.

Potato Dextrose Agar (PDA) was used for the cultivation of fungi, and nutrient agar was used to cultivate bacteria. Microbial infections were identified using light microscopy after their cultivation.

The types of fungi and bacteria: The mold fungi were *Trichoderma*, *Acremonium SP*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium SP*, while the bacteria were *Micrococcus sp. (G+)*, *Micro Bacillus sp. (G+)* and *Short Bacillus sp. (G+)*. The fungi and bacteria were on wood and color, while black resin any fungi, and bacteria were absent because the black resin contained anti-fungal and anti-bacterial materials.

KEYWORDS: Polychrome wooden coffin, black resin, Bio-deterioration, Fungi, Bacteria

1. INTRODUCTION

Wood is a natural organic material that consists of hemicellulose, cellulose, lignin, and minor amounts of extraneous materials, mostly in the form of organic extractives and inorganic minerals, such as ash (Mansour *et al.*, 2015). It is usually very sensitive to biological attacks. Therefore, fungi, bacteria, and insects can easily attack and metabolize it, leading to significant physical, chemical, and morphological changes. Microorganisms, with other deterioration factors, can be responsible for the destruction of cultural heritage and make a change in the chemical structure of archaeological materials.

All historical wooden objects are at risk from insect and fungal attacks (Hunt, 2012) and biological degradation (Walsh *et al.*, 2014). Fungi play a considerable role in the deterioration and degradation of cultural heritage due to their enormous enzymatic activity (Sterflinger., 2010). Most of the studies focus on the biodeterioration of valuable wood objects damaged by fungi and bacteria. Furthermore, fungi are very often found in the display and storage conditions of museums (Abdel-Kareem, 2010). In evaluating the efficiency of many consolidation materials used for consolidating the black resin samples, see (Abdelmoniem *et al.*, 2020).

It was found that low temperatures and decreasing oxygen concentrations inhibit the action of all fungi and bacteria (Tamburini *et al.*, 2017). However, wooden artifacts covered with black resin differ from other wooden artifacts in resisting microbial damage.

A lot of wooden artifacts were covered with a black resin layer in the New Kingdom from 1471 BC and continued to the next age until the Ptolemaic era 30 BC (Abdelmoniem, 2019). The present study aims to prove that black resin had some antifungal and antibacterial materials and insect repellents, such as mastic resin, colophony, beeswax, and bitumen. These results help make a plan for the conservation process of the coffin.

2. MATERIALS AND METHODS

Isolation and identification of fungi and bacteria

Samples were taken from the different parts of the object dating back to 664–525 BC. Then, cotton swabs were pressed firmly over the surface of the object. Swabs were placed in a sterile tube (Elserogy *et al.*, 2017). Five samples were utilized: (Fig. 1) one for wood, one for color, two samples for black resin,

and one for the surrounding air of the coffin (Abdelmoniem, 2019).



Figure 2: Locations of microbial swabs; 1) Wood; 2) Painted layer; 3&4) Black resin; 5) Surrounding air of the coffin

Cultivation method The samples were isolated and purified. The identification of fungi and bacteria was conducted at the Microbiology Laboratory (Zidan *et al.*, 2016), Faculty of Agriculture, Fayoum University. PDA was used for the cultivation (Aryal, 2019), isolation, and growth of a wide range of fungi (Uthayasooriyan *et al.*, 2016; Ravimannan *et al.*, 2014).

The media used to grow fungi The samples were cultivated on PDA. The media were prepared in the following proportions: Potato starch (100 g), Agar (10 g), Glucose (10g) (Atlas, 2010), and distilled water (500 ml). The nutrient medium was used for the cultivation of microbes supporting the growth of a wide range of non-fastidious organisms (Aryal, 2019; Venturini Copetti *et al.*, 2009). The plates were incubated at 28°C for 5-7 days. Then, they were examined at the Microbiology Laboratory, the Grand Egyptian Museum to diagnose the type of microbial infection.

The media used to grow bacteria The samples were cultivated on nutrient agar (Uthayasooriyan *et al.*, 2016). The media were prepared in the following proportions: Peptone (5 g), agar (10 g), beef extract (Atlas, 2010) (1.5g) and distilled water (500 ml). The

samples were incubated at 28 °C, and their growth was observed on the fourth day. Microscopic slices were made to identify the type of bacteria.

The light microscopy (Zeiss Axio Imager .MI, equipped with Axio Cam MRC5) was used to identify the fungi and bacteria.

3. RESULTS

There is no microbial growth on the black resin layer because it contains anti-fungal and anti-bacterial materials (Tables 1, 3).

3.1 Identification of fungal isolation

The results of (Table 1) refer to the total absence of fungi in the samples C-D due to the presence of some anti-fungal substances in the black resin. Sample E was taken from the surrounding air of the coffin, while sample A (coffin wood) showed the highest level of fungi.

The following types of fungi were identified: *Trichoderma*, *Acremenium SP.*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium SP* (Table 2) (Fig. 2) from the wood and color of the coffin.

Table 1. Total Count Of Fungi

Sample	Wood			Color			Black resin			Black resin			Air		
Symbol	A			B			C			D			E		
Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³
I	8	--	2	2	1	1	--	--	--	--	--	--	--	--	--
II	19	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Mean	13.5	--	1	2	0.5	0.5	--	--	--	--	--	--	--	--	--

Table 2. shows the names of the identified fungi

sample	fungi	Symbol	Dilution						
			10 ⁻¹		10 ⁻²		10 ⁻³		
			I	II	I	II	I	II	
Wood	Trichoderma	A							
	Acremenium SP.								
	Aspergillus niger								
	Penicillium SP.								
Color	Aspergillus flavus	B							
	Aspergillus niger								

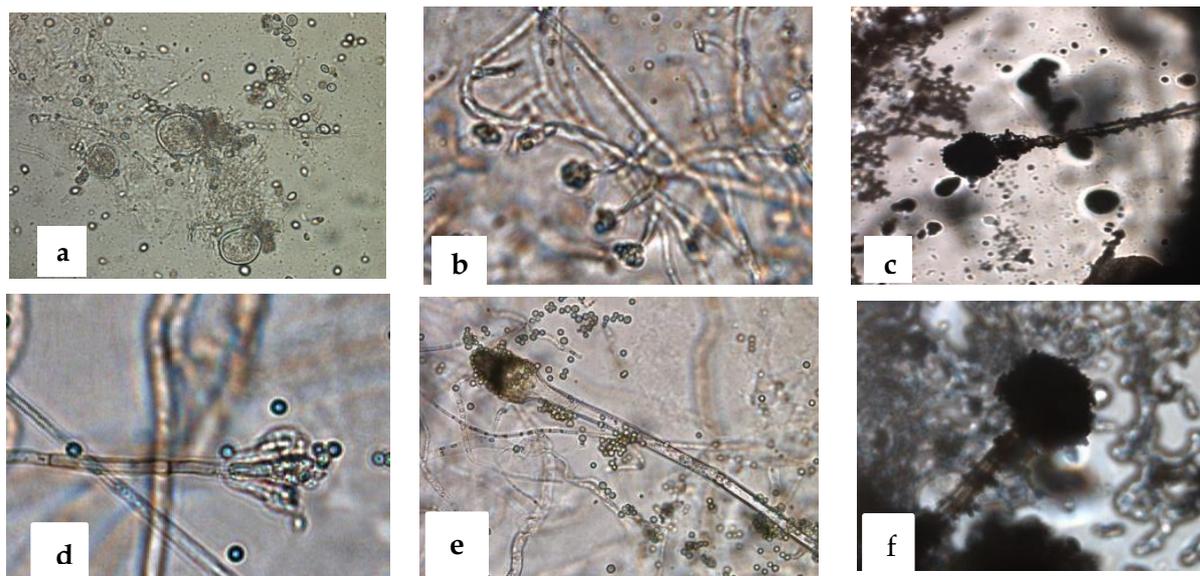


Figure 2. The fungi isolated from wood and the color of the coffin; a) Dilution A10⁻¹ II *Trichoderma Persoon ex Grey*; b) Dilution A10⁻³ I *Acremenium SP.*; c) Dilution A10⁻¹ I, *Aspergillus niger*; d) Dilution A 10⁻³ I *Penicillium SP.*; e) Dilution B10⁻¹ II *Aspergillus flavus*; f) Dilution B10⁻² II *Aspergillus niger*

3.2 Identification of Bacterial isolation

The results of bacteria numbers in (Table. 3) almost showed results similar to those of the fungi's. The numbers of bacteria were more on the wood of the coffin, but the color was less on infection than wood. Black resin samples did not show any growth in the samples because black resin contains anti-

fungal and anti-bacterial materials, such as mastic resin, colophony, beeswax, and bitumen (Abdelmoniem et al., 2019). These materials protect the black resin layer from microbial infection.

The following types of bacteria were identified: *Micrococcus sp. (G+)*, *Micro Bacillus sp. (G+)*, and *Short Bacillus sp. (G+)* (Table 4) (Fig. 3) from the wood and color of the coffin.

Table 3. Total Viable Count (TVC) of Bacteria:

Sample	Wood			Color			Black resin			Black resin			Air		
Symbol	A			B			C			D			E		
Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻¹	10 ⁻²	10 ⁻³
I	12	--	--	1	1	--	--	--	--	--	--	--	--	--	--
II	14	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Mean	13	--	--	1.5	0.5	--	--	--	--	--	--	--	--	--	--

Table 4. showing known bacteria

slide	sample	bacteria	symbol	Dilution							
				10 ⁻¹		10 ⁻²		10 ⁻³			
				I	II	I	II	I	II		
Slide 1,2	Wood	<i>Micrococcus sp. (G+)</i>	A								
Slide 3, 4		<i>Micro Bacillus sp. (G+)</i>									
Slide 5,6,7		<i>Short Bacillus sp. (G+)</i>									

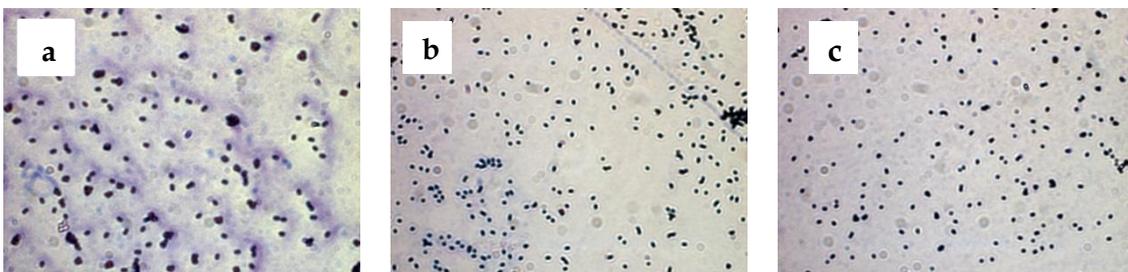


Figure3. the bacteria isolated from wood of the coffin; a) *Short Bacillus sp. (G+)*; b) *Micro Bacillus sp. (G+)*; c) *Micrococcus sp. (G+)*

4. DISCUSSION

The sample of coffin wood showed the highest level of fungi numbers that may be due to the secretion of these fungi of the enzyme analyses of the cellulose. The fungi of the conventional microbiological examination are shown in Table 2.

In this context, it must be pointed out that despite the emergence of these microbes (Table 3), the figures are negligible as a source of damage and contamination for the emergence of higher setting in mitigation 10⁻¹ that is a low number when compared to other bacteria numbers, which may appear on some artifacts that may be contaminated due to high humidity (Abdelmoniem., 2019).

Microbial deterioration causes many deterioration forms, e.g. macro- and micro-cracks, hydrated salts, flaking, coloration, scaling, and defoliation

microbiological spots (Geweely et al., 2014). Thus, the deterioration of wood on account of microbiological agents is an acknowledged fact (Darwish et al., 2013).

The layer of black resin enters, in its complexes, a group of natural resins and other substances whose properties are anti-fungi and bacteria. Therefore, the black resin has no microbial infestation.

5. CONCLUSION

Fungi play a very important role in the deterioration of wooden artifacts. In this study, the fungi isolated from the coffin were *Trichoderma*, *Acremonium SP.*, *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium SP.*, whereas the bacteria were *Micrococcus sp. (G+)*, *Micro Bacillus sp. (G+)*, and *Short Bacillus sp. (G+)*. The black resin had some anti-fungal and anti-bacterial, as well as insect repellent

components. Thus, no organism could be found on it. The microbial examination shows the type of injury that affects the wooden artifacts in order to choose the best methods for sterilization and treatment. The study proves that the black resin material has anti-fungal and bacterial substances.

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