



## Analytical Techniques for Condition Assessment of Leather Binding of A Historical Manuscript from El-Azhar Al-Sharif Library

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### Abstract

Leather artifacts in El-Azhar El-Sharief Library suffer from deterioration. Some aspects of deterioration are found on these artifacts such as darkness, missed parts, insect holes, etc. These are due to the effect of some factors (physical, chemical, biological, and human factors). This study aims to evaluate the condition of a leather binding, explain the aspects and mechanism of its deterioration, and prepare for laying a plan for applying the appropriate conservation techniques. The analytical techniques used were visual assessment, digital microscope, investigation of the surface morphology by scanning electron microscope (SEM), Fourier Transform Infrared (FTIR), pH value, and isolation and identification of fungi. The digital light microscope revealed that the surface became coarse, and advanced erosion was also noticed. SEM revealed the weakness of the fiber structures of leather, and holes caused by insects were also noticed. FTIR analysis proved that there was a change in the chemical composition of historical leather. The identified fungi were *Aspergillus* sp. Ah55. pH measurement stated that the pH of leather binding decreased significantly under the effect of acidic conditions in the surrounding environment conditions. This study recommends the necessity of carrying out conservation treatment processes for the leather binding studied including cleaning, consolidation, disinfection, completion of missed parts, etc. Preventive conservation should be also taken into consideration.

**Keywords:** Leather binding, deterioration, Visual assessment, microscopes, pH value, FTIR, Genotypic study

### 1. Introduction

Leather artifacts are found in Egyptian museums and storehouses in public and private libraries. Leather is a protein called collagen [1]. Collagen is a fibrous protein [2], formed by the linking of amino acids. About twenty amino acids occur in nature that combines in different formations to form all proteins.

Leather artifacts in the different locations especially at Al-Azhar Library, in Cairo, Egypt are often exposed to many deterioration factors in these places. There is fluctuation in relative humidity and temperature, and exposure to access light. This physical factor can lead to cracks in leather, and a change of color [3, 4]. It also plays an important role

in the deterioration mechanisms by hydrolysis, oxidation, or both of them [5]. The chemical degradation caused by pollutants and particulates also plays a role in the acid hydrolysis mechanism and causes a reduction in the pH value [6]. It can be added that the biological factors of insects and microorganisms play a major role in the degradation process caused by acids or enzymes produced by them, which react with the chemical composition of leather. Sometimes, biological factors can lead to the total conversion of leather from collagen to gelatin with an advanced state of degradation or denaturation [7-9]. The inappropriate handling of the manuscripts may also play an important role in the

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degradation process. The authors argued that the studied leather binding was exposed to all the factors mentioned above [10].

Accordingly, many aspects of deterioration are found in the studied leather binding such as accumulated dust, erosion, stains derived from different sources, fragility, weakness, etc. It can be said that these aspects of deterioration may be due to the inappropriate environmental conditions in which the manuscript was located or the places in which the studied manuscript was stored before its coming to the current place [8, 9].

It was recommended that the condition assessment of any archaeological materials is considered the most important point to take an account about the degradation process in order to produce some solutions for the conservation treatment or preventive conservation. Accordingly, the analytical techniques used in this study supply insights into the raw materials used in the manufacturing process of the leather, its internal structure, the material history, and the degradation mechanisms of the studied leather binding, and are key in developing mitigating strategies for a broad range of conservation issues [11-18].

Visual assessment by digital camera with the assistance of the critical eye of the authors, was applied to determine the aspects of deterioration found on the studied object. This method is important because, through the accumulation of knowledge and experience, the authors can predict the factors of deterioration and explain its mechanics. The critical eye of the conservator can also determine the most effective techniques of analysis, which should be applied to evaluate the condition of the leather binding studied [19, 20].

The use of a scanning electron microscope for investigation of the surface morphology of leather is considered an important technique for observing the morphological characteristics of the deteriorated leather at the level of grain pattern, evaluating their deterioration, and identifying the causal agent of decay patterns [21].

ART/FTIR analysis is considered one of the most important techniques used to characterize the composition, and structure molecules of leather, and give details about the deterioration process. It is easy to use, relatively inexpensive, and non-destructive method [22].

In the conservation context, pH is a key parameter for detecting the state of preservation of an object. It is very important for historical leather artifacts especially those found in polluted areas such as the studied object [23].

The identification of fungi is important to know their types and explain the deterioration caused by them.

It also gives information on how the conservator makes a plan for monitoring the process [24].

This study aims to use some analytical techniques to identify the materials used in the historical leather binding of the manuscript and to explain its aspects and mechanism of its deterioration in order to be produced to the decision maker or to be used for making a plan for the conservation process in the next study.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Preparation of new vegetable tanned leather

New vegetable goat-tanned leather samples were prepared according to Abdel-Maksoud [25, 26].

#### 2.1.2. Leather binding studied

The studied leather binding is stored in El-Azhar Al-Sharif Library with general No. 85892, and special No. 3080. The date of the manuscript is unknown, but by comparison of the decoration on the leather binding of writing on the paper sheets of other manuscripts, it approximately dates back to the period from 1140 AH - 1180 AH. The length of the manuscript is 22 cm, and the width is 15.5 cm.

### 2.2. Analytical techniques used

#### 2.2.1. Photographic documentation (visual assessment)

The visual examination was done using the naked eye after documentation and photography using camera 16 Megapixels. This technique was used to document the aspects of deterioration found in the studied leather binding [10, 19].

#### 2.2.2. Digital light microscope

The portable USB digital microscope (model PZ01-Shenzhen Super Eyes Co. Ltd., China) was used to examine the surface of the new and historical leather samples [27, 28].

#### 2.2.3. Scanning electron microscope (SEM)

A scanning electron microscope used in this study for the investigation of the surface morphology was Zeiss LEO 1550 (SEM LEO 1550VP; Carl Zeiss AG, Oberkochen, Germany), equipped with an Edwards Scan Coat K550X sputter coater (Gordon Brothers, Boston, MA, USA) at Asyut University [29].

#### 2.2.4. Measurement of pH value

The measurement was performed directly on the aqueous cold extraction using AD11 Romania waterproof pH tester; combined with an electrode and calibrated to between 2 and 7, at 21–22 °C.;

Very small pieces of samples of about 0.2 g were taken mechanically of loose fibers of grain layer from new and historical leather binding [30, 31].

### 2.2.5. Fourier transform infrared spectroscopy (FTIR)

The molecular structure of the studied samples was investigated using Fourier transform infrared spectroscopy (FTIR) on BrukerOptik GmbH Vertex 70 FTIR Spectrometer equipped with DLaTGS detector by recording 32 scans, in the range of 4000–400  $\text{cm}^{-1}$  with spectral resolution of 4  $\text{cm}^{-1}$  [32].

### 2.2.6. Isolation and identification of endophytic fungi

From the falling samples of the examined leather binding, very tiny samples were obtained and put on a potato dextrose media plate with the following composition: distilled water, potato extract (4.0 g/L), dextrose (glucose) (20.0 g/L), and agar (15.0–20.0 g/L) (used as a solidifying agent). 1,000 mL (1 L) of water. The plates were kept in an incubator (from 7 – 10 days) at 28 degrees Celsius until endophytic fungal growth was seen. To obtain pure culture with a variety of morphological features, colonies were selected, subcultured on a new medium using Potato Dextrose Agar (PDA) without the use of antibiotics, and then kept at 4°C. The National Research Center (NRC) in Egypt's Microbial Chemistry Department is home to strain AH55.

#### Genotypic study

The 18S rDNA sequencing of the fungal isolate was analyzed for additional identification confirmation. In order to extract fungal DNA, mycelia were infected and grown for 4 days at 28 °C in a 250 mL Erlenmeyer's flask containing 50 mL of potato dextrose broth medium. Following the incubation time, genomic DNA was extracted from the mycelial biomass using the QiagenDNeasy Mini Kit according to the manufacturer's instructions. The 18S rRNA gene amplification studies were carried out using two universal primers, NS3 (5'-GCAAGTCTGGTGCCAGCAGCC amplification. 3') and NS4 (5'-CTTCCGTC AATTCC TTTAAG-3') [33]. In the PCR temperature profile, a 5-minute denaturation step at 94 °C was followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 90 seconds, and a final extension step at 72 °C for 5 minutes. The amplified products were electrophoretically analyzed and sequenced at the SolGent Company in South Korea. Using the BLASTN program, the produced sequence was compared to other similar sequences in the NCBI database (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). Phylogenetic tree created with MEGAx program. The evolutionary history was deduced using the

Maximum Likelihood method and the Tamura-Nei model. The displayed tree has the highest log probability (-22507.44). The percentage of trees containing related taxa is shown next to the branches. The first tree or trees for the heuristic search were generated automatically using the Neighbor-Join and BioNJ algorithms on a matrix of pairwise distances estimated using the Tamura-Nei model. After that, the topology with the highest log-likelihood value was chosen. The tree is depicted to scale, and branch lengths are given in terms of the number of substitutions per site. This study includes eighteen different nucleotide sequences. The positions of the first, second, third, and noncoding codons were assigned. The completed dataset includes a total of 1504 locations. MEGA X was used for evolutionary analysis.

## 3. Results and discussion

### 3.1. Photographic documentation (visual assessment)

The data obtained (Fig. 1) showed that the studied leather binding suffers from advanced degradation. The following aspects of deterioration were found on the front and back sides of the leather binding:

❖ **Insect holes:** insect damage was observed on the surface of the front and back sides of the leather binding. This damage may be due to unsuitable environmental conditions in its current and previous storage environments. Insect damage is considered one of the most serious damage factors facing historical leather in unsuitable preservation environments in museums and libraries, where there is a great frequency in temperature and is an organic material that represents a suitable food source for insects and microorganisms, due to the fact that it contains carbon, which provides insects with the energy necessary to carry out their activities. Leathers are nutrients for many species of insects, including dermestids (from the carpet beetle family) [34]. It can also be said that insects produce acids or enzymes that react with collagen (amino acids) [9], and degradation in the form of holes is obtained at the final step of this reaction.

❖ **Accumulated dust:** accumulated dust covered the surface of both sides of the leather binding. This may be due to an uncontrolled environment, especially for the chemical factors derived from air pollution and particulates. The problem with this dust is that it can contain some elements such as copper or iron, which can assist in the conversion of sulfur dioxide into sulphuric acid, which is considered the most dangerous one for leather and related materials. This dust can also contain eggs of insects, or spores of fungi, and cause microbiological deterioration for leather artifacts. It can be added that accumulated dust on the surface of the leather with increasing

relative humidity in the surrounding environmental conditions plays an important role in the formation of hard crust stains, which act as a big problem for conservators to remove.

❖ **Missed parts:** It was noted some missed parts on the edges of the leather. This was due to the weakness of mechanical properties as a result of the unsuitable environmental conditions in different storage locations of the leather binding such as fluctuation in relative humidity and temperature, excess light, etc. Improper handling also plays an important role in causing this aspect of deterioration.

❖ **Erosion of tanning material:** The erosion of the tanning material was noticed in different places on the surface of the manuscript. This was due to the friction of the manuscript with some other solid materials. Another reason for this aspect of deterioration was due to the weakness of the finishing layer (adding insufficient lubricants) in the final stage of leather tanning. It can also be due to the effect of microorganisms and insects.

❖ **Non-homogeneity of the color:** This may be due to the heterogeneity of the tanning material during the manufacturing process, or it may be due to the heterogeneity in exposure to the deterioration factors in the surrounding environment conditions.

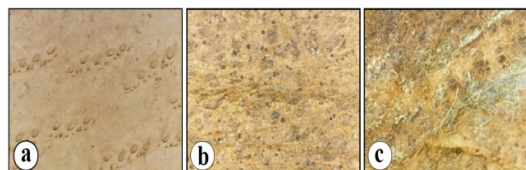
❖ **Loss of inner lining:** The effect of surrounding environmental conditions (physical, chemical, and biological factors) led to this aspect of deterioration. Choosing an unsuitable type of paper for the inner lining may helped to cause this aspect of deterioration.



**Figure 1:** The front and back sides of the historical leather binding studied

### 3.2. Digital light microscope for investigation of the leather surface

It was clear from the data obtained (Fig. 2) that the surface of the control sample (Fig. 1a) was smooth, and the grain surface pattern was distinct from goat skin. For the historical leather binding (Fig. 1b and 1c), the grain surface pattern was recognized and indicated that the goat skin was used in the manufacturing process of the leather binding. The surface became coarse. Some insect holes appeared on the leather surface. The data also showed the erosion of tanning material. Deformation of some parts was also noticed.



**Figure 2:** Digital microscope investigation of new and historical vegetable-tanned leathers: (a) control sample, (b and c) historical binding

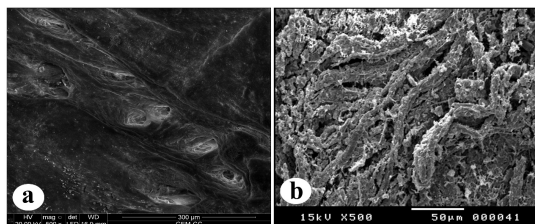
### 3.3. Investigation of the surface morphology by scanning electron microscope

It was clear from the SEM investigation of the control sample (Fig. 3a) that the surface was smooth. The surface pattern is characteristic of goat skin. The grain surface layer is also present regularly on the surface. For the historical sample (Fig. 3b), the grain surface is indistinguishable, and the type of animal skin is not identified. The effect of environmental conditions on the surface was observed through uneven surface, roughness, fractures, tears, and random distribution of fibers. The presence of mycelium in some fungi has also been observed. Accordingly, the historical leather was in bad condition, and needed some treatments to improve its properties such as mechanical properties, and surface appearance. As shown in Fig. 3b, It can be observed that the disintegration occurring between the fibrous cells of the leather furthermore the grain layer had been destroyed completely. In addition, mycelium threads were observed spreading among the leather structure; and this is tangible evidence of a fungal infection of the leather binding.

### 3.4. Measurement of pH value

The measurement of pH value is one of the important studies that many researchers relied on in the field of leather treatment and conservation in order to determine the type and severity of infection depending on the pH [34].





**Figure 3:** Investigation of the surface morphology by a scanning electron microscope of new and historical leather: (a) New sample; (b) Historical sample

Furthermore; determining the optimal method of treatment without damaging the leather surface. The optimum pH value of leather after the tanning process should be between 4-6 [35, 36]. This pH value ensures that the tannins and fat bound in the leather remain. Under the impact of various degradation factors, the pH value changes either by increasing (alkalinity) or decreasing (acidity), according to the nature of the degrading factor. The data obtained showed that the pH value of the new vegetable-tanned leather was 5.1 pH, while the studied leather binding was 2.2. This value is considered high acidity.

Acidic degradation of leather is a chemical process in which there are a great number of contributing factors such as pollution, heat, heavy metals, and oxygen. All these factors; in combination with the complexity of the leather and tanning chemistry, combine to give complex and diverse deterioration mechanisms.

Vegetable-tanned leather under acidic conditions usually suffers from two mechanisms oxidation and hydrolyses [37-39]. Actually, the historical leather under the current study had been stored in the El-Azhar library which overlooks a road that includes different forms of transportation. Therefore the environment in this region is polluted with the sulfur dioxide, which absorbs and transforms it into sulphuric acid which in turn the moisture in the leather dissolves into positive hydronium ions [40, 41]. As a result of the deterioration by high levels of pollution for example sulfur dioxide, a phenomenon called red rot will often appear. The leather will crumble into a powder, reddish brown in color [42-44].

In addition to the pollution present in the Al-Azhar area where the manuscript is preserved, there are other factors that reduce the pH value of the skin. One of these factors is the excessive use of acids in high concentrations during the tanning process. Acids secreted by microorganisms and insects also play a major role in reducing the pH value.

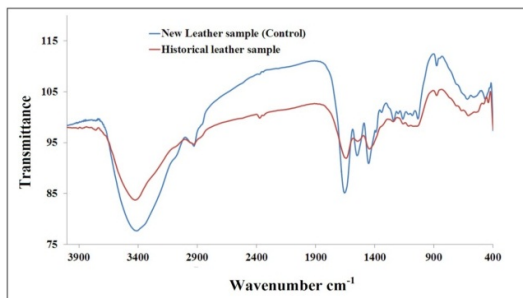
### 3.5. Attenuated total reflection – Fourier transform infrared spectroscopy (ATR/FTIR)

The data obtained from Fig. 4 for the control sample showed the observable typical bands of leather protein e.g. Amide A at  $3300\text{ cm}^{-1}$  which is associated with stretching of peptide N-H groups involved in inter-chain hydrogen bonding beside that Amide I at  $1631\text{ cm}^{-1}$  corresponding to C=O stretching vibration of peptide bonds along the polypeptide backbone with a small contribution from N-H in-plane bending; Amide II at  $1536\text{ cm}^{-1}$ ; associated with N-H bending and C-N stretching vibration, finally, Amide III at  $1232\text{ cm}^{-1}$  which associated with N-H in-plane bending and  $\text{CH}_2$  wagging vibration of glycine backbone and proline side chain.

By comparing the spectra between the historical and control one we can note significant differences between the two samples such as the shifting peaking in regions about  $2000\text{-}1000\text{ cm}^{-1}$  (C–O and C–C stretching), scientifically, phospholipid was characterized by absorption peaks at  $1000\text{ to }2000\text{ cm}^{-1}$  [45, 46] in the historical sample. Furthermore, the intensity of Amide I (CO stretching) at  $1636\text{ cm}^{-1}$  in historical leather was higher than standard tanned leather. Simultaneously, the intensity of vibrations of C–N–H bending at  $1536\text{ cm}^{-1}$  was lower in the control sample. OH stretching bands at  $3413\text{ cm}^{-1}$  were displaced to wave numbers at  $3423$  and  $3411\text{ cm}^{-1}$  for historical and control samples, these changes can be attributed to the effect of deterioration, and natural aging over time. Usually, the leather fibers derive from the extended conformation of the triple helix molecule that is cross-linked with hydrogen bonds; a portion of those bonds is heat sensitive and then breaks down when collagen is exposed to a range of temperatures over time. Disrupting these stabilizing hydrogen bonds releases the collagen chains and results in triple-helix collapse and the formation of a random-coil structure. This process is called thermal denaturation. Therefore, when leather is subjected to heat aging beyond its maximum tolerance, its three-dimensional molecular structure denatures, and all the macro-structures and micro-based on it collapse. The numerous polar groups present in tropocollagen molecules [47], besides atmospheric pollutants, promote various deterioration reactions, especially in association with water. Furthermore, Sulfur ions are the most harmful factors to cause oxidation. Even dust particles can increase chemical and physical damage by absorbing water vapor and pollutants, and finally by the existence of microorganisms.

Furthermore, water molecules can bind, making leather a hygroscopic material: it absorbs, retains, and releases moisture as it seeks balance with its environment. Due to these intrinsic characteristics of

collagen leather is very sensitive to humidity and it can cockle, wrinkle, curl, and distort in moisture-heavy environments. respectively the dislocation of OH stretching can be attributed to producing H-H banding and forming vacancies of oxygen in the internal molecular of collagen [48, 49]; moreover; the result illustrates that the intensity of C-H bending at  $2900\text{ cm}^{-1}$  (tetrahedral carbon-hydrogen bonds) [40, 51] drastically reduced in historical sample Which may indicate that the archaeological sample lost a high amount of fat.

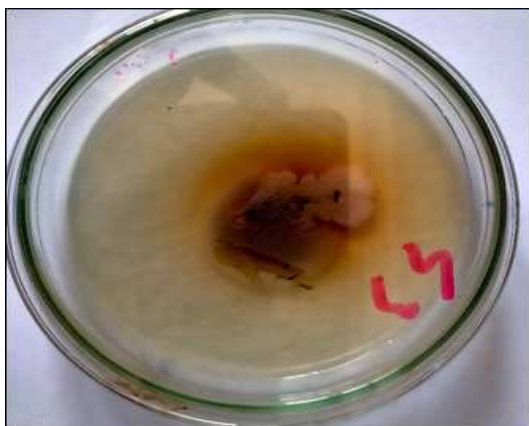


**Figure 4:** ATR-FTIR analysis of new and historical vegetable tanned leathers

### 3.6. Isolation of the producing endophytic fungal strain

To get the fungus Ah55, samples were taken from the leather binding of a medieval manuscript at the Al-Azhar Library in Egypt (Fig. 5). A potato dextrose agar plate was inoculated with the material, and after 15 days of incubation, the plates were examined and fungal colonies were identified. The strain was placed in the Microbial Chemistry Department of the National Research Centre in Cairo, Egypt.

Abdel-Nasser et al. [ ] reported that fungi are among the most harmful species that contribute to the



**Figure 5:** Representative photos fungal Ah55 Mycelium on PDA

biodegradation of historical organic materials including books, manuscripts, and book bindings made of vegetable-tanned leather. They also added that the penetration of fungal hyphae in the fibers can cause mechanical stress on the components of the manuscripts weakening them, and they secrete extracellular enzymes, leading to biodeterioration [52]. The results were also confirmed by some authors [53, 54] who reported that Fungi are considered the most dangerous for organic materials, which may be due to enzymes or acids produced by them, which lead to physical or chemical changes.

### Molecular Identification

Using the BLAST tool (<http://www.blast.ncbi.nlm.nih.gov/Blast>), the 18S rRNA gene sequence was obtained, identified, and compared to other known sequences in the GeneBank database to ascertain the similarity score and statistical significance of the hits. *Aspergillus* sp. and isolate Ah55 showed 100% homology, and the obtained result showed a striking similarity to the 18S rRNA gene sequence. According to Kumar et al. [51], the phylogenetic analysis and tree were created using MEGA x software and the Maximum Likelihood approach. Based on physical features and DNA sequence analysis, the strain Ah55 was identified as *Aspergillus* sp. Ah55. Its accession number, OL822325.1, has been deposited in GenBank (Fig. 6).

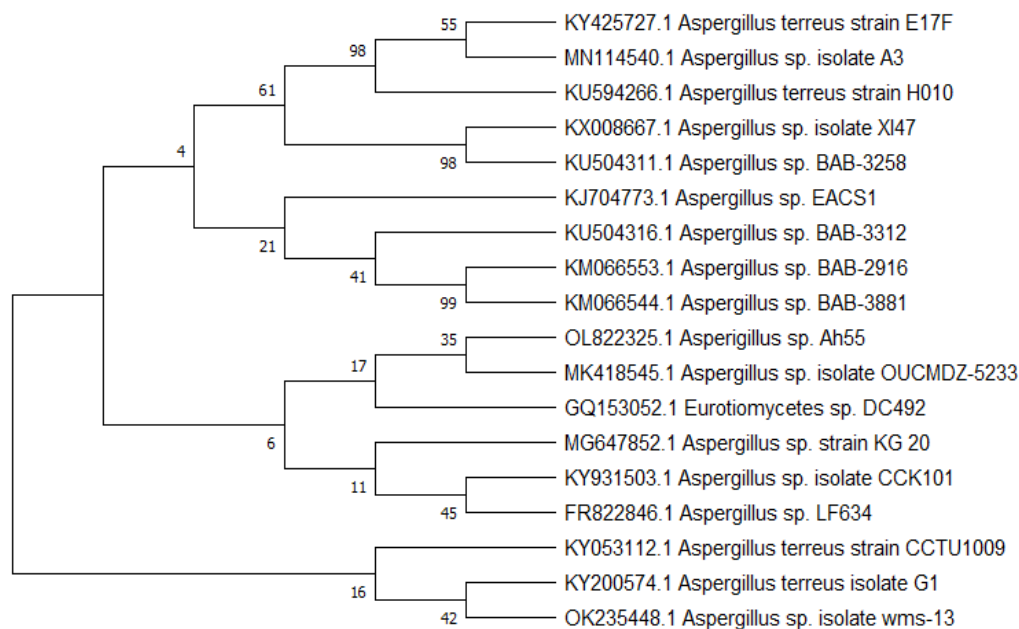
The analysis of the 18S rRNA gene sequence of microbial isolate Ah55 yielded important results that shed light on its taxonomic identity and genetic relationships. The primary goal of this study was to determine the identity of isolate Ah55 at the molecular level. To achieve this, researchers retrieved the 18S rRNA gene sequence and compared it with known sequences in the GeneBank database using the BLAST program. The outcome was a perfect 100% homology match with *Aspergillus* sp., definitively categorizing Ah55 within the *Aspergillus* genus. Furthermore, the high degree of homology observed in the BLAST analysis signifies an exact correspondence between the 18S rRNA gene sequence of Ah55 and reference sequences of *Aspergillus* sp. This level of similarity carries significant statistical weight, firmly supporting the conclusion that Ah55 belongs to the *Aspergillus* genus.

In addition to confirming the taxonomic identity, researchers conducted a phylogenetic analysis to explore the evolutionary relationships of Ah55 within the *Aspergillus* genus. Employing the Maximum Likelihood technique, a phylogenetic tree was constructed based on the 18S rRNA gene

sequence, following the methodology outlined by [55]. This phylogenetic tree likely included closely related *Aspergillus* species, providing a visual representation of Ah55's genetic relatedness to other members of its taxonomic group. To make this valuable genetic information widely accessible, the 18S rRNA gene sequence of isolate Ah55 was deposited in the GenBank database and assigned the unique accession number OL822325.1. This accession number serves as a specific identifier for this genetic sequence, facilitating access and reference for researchers in their own work.

It can be said that historical leather is often susceptible to degradation caused by fungi due to

their organic nature and susceptibility to environmental conditions [56-61]. Fungi, particularly mold and mildew species like *Aspergillus* and *Penicillium*, thrive in environments with high moisture levels, breaking down the collagen and other compounds that give antique hides their structural integrity [62]. The impact of fungal infestations on these artifacts can be devastating, leading to discoloration, weakening of the hide, and the potential for spore dissemination, posing a significant risk to the aesthetic and structural qualities of the antique hides [63].



**Figure 6:** Constructed tree using Neighbour-Joining method to match the fungus Ah55 to already published sequences

#### 4. Conclusion

This study focuses on the leather binding of a historical manuscript deposited in the El-Azhar Al-Sharif Library. The visual assessment by the critical eyes and digital light microscope revealed that the leather binding suffers from adverse deterioration, which may be due to a combination of factors (physical, chemical, and biological). Accordingly, some aspects of deterioration appeared such as insect holes, accumulated dust, missed parts, erosion of tanning materials, etc. The use of microscopes (digital and SEM) proved that the skin used in the manufacturing process of new and historical leather binding was from goat skin. The microscopes also proved that some aspects of deterioration appeared

on the historical leather binding such as coarse of the surface, erosion, etc. The pH value of the historical leather binding was 2.2 pH, which was considered low and was one of the main factors that led to the deterioration of the studied object, especially by the acid hydrolysis mechanism. ATR-FTIR analysis proved that there were some changes in the functional groups (such as amide I, and amide II) in the studied object compared to the new vegetable-tanned leather sample. This indicated that some changes occurred in the chemical stability of the historical sample. These changes may be attributed to the improper factors in the surrounding environmental conditions of the object. The strain

AP5 isolated from the studied object was identified as *Aspergillus sp.*

This study recommended that the studied leather binding needs conservation processes urgently. It requires conservation treatment including cleaning, consolidation, gap filling, disinfection, etc. It also requires preventive conservation to control the surrounding environmental conditions (temperature, relative humidity, light, air pollution, microorganisms, insects, etc.).

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