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ISOLATION, CHARACTERIZATION AND TREATMENT OF MICROBIAL AGENTS RESPONSIBLE FOR THE DETERIORATION OF ARCHAEOLOGICAL OBJECTS IN THREE JORDANIAN MUSEUMS

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ABSTRACT

This study aims at isolating, identifying and appropriately treating of archeological objects in Jordanian Museums that were attacked by microorganisms.
Archeological objects (organic and inorganic) conserved in three Jordanian Museums included: The Museum of Jordanian heritage; Faculty of Archaeology and Anthropology at Yarmouk University (YU), Dar as-saraya Museum in tallirbid (DS) and Museum of archeology, Jordan University (JU). Fifty samples were analyzed.
Results indicate the presence of 8 fungal species on tested archeological objects. Furthermore, the identified bacterial isolates were found to be referring to 11 bacterial species. Of these 19 objects 5 were found to be inhabited by the fungus *Penicillium chrysogenum*, 7 objects inhabited by *Aspergillus niger* (the black mould) and 8 objects were contaminated by yeast. The fungus *P. chrysogenum* was found to inhabit mainly bones, wooden objects and clay pots. Yeast was found in bones, snail shells, wood, iron and textiles. However, *A. niger* was contaminating bones, wooden objects, clay pots, unbaked clay jars and basalt.
The *pseudodiphtheriticum* was found on bones, pottery, stones, bronze and glass objects, whereas, *C. aquatium* inhabited mortar, stones, bronze and glass. 11 archaeological objects at the archaeological Museum, University of Jordan, were found to be contaminated by 10 bacterial and only one fungal species which is yeast. However, of these 11 objects 9 were found to be inhabited by the yeast fungus and these objects were represented by bones, antiler horn, wood, horns, plaster floor, bronze, glased pottery. Finally, 6 objects representing antiller horn, wood, glased pottery and bone were contaminated by *C. aquatium*, whereas, 4 objects (bone, horns, wood and albaster) were contaminated with *Corynebacterium pyogenes*. Furthermore, 7 objects were contaminated with *Listeria monocytogenes* and these 7 objects include wood, plaster floor, pottery, albaster and glased pottery.

KEYWORDS: Jordanian museums, microbial, bacterial, fungal, yeast, contamination, archaeological objects

1. INTRODUCTION

Archaeology is the study of past human societies, through recovering, analyzing material culture and reconstructing past life ways. The preservation and management of past human physical remains would help in keeping the archaeological object of the distant future (Hardesty, 2007).

Microorganisms can be responsible for the destruction of cultural heritage, together with several environmental conditions, ageing and the chemical structure of substrate (Griffin, 1991; Bock, 1993; Cifferi, 1999).

Microorganisms played major roles in deterioration of archeological objects whether these archeological objects are of organic or inorganic nature. The growth of microorganisms on oil painting, mural painting and frescos causes several aspects of the deterioration and these include spotting, discoloration and detachment layer. There are some types of microorganisms that produce visible and viscose layer (biofilm). This biofilm helps in adhesion of dust particles and pollutants from the air (Milanesi 2006). Hence, microorganisms cause damage to antiques, these microbes could lead to deterioration either by feeding on archeological object or through secreting enzymes that would lead to erosion and corruption of antiques. The factors which might influence the microbial growth on archeological object are mainly environmental factors such as temperature, humidity and pH, this is in addition to the type of archeological object whether it is organic or inorganic.

In this research work we aimed to study the effect of microbiological activities and their impact on the deterioration of archeological objects.

The word microbiology is a broad term meaning the study of living organisms that are individually too small to be seen with the naked eye. It includes the study of bacteria (bacteriology), viruses (virology), yeasts and moulds (mycology). Such forms of life are given the name microorganisms (Volk,1992).

Bacteria are very different from fungi; bacteria are prokaryotes and much smaller in size. Bacteria can be found in three basic shapes: Coccus (spheres), Bacillus (rods) and Spirillum (spirals) (Foster, 2012). Most bacteria grow best around neutral pH values (6.5 - 7.0), but some thrive in very acid conditions and some can even tolerate a pH as low as 1.0. Some bacteria produce acids and other metabolites as they grow. The accumulations of acidic metabolites have several effects on the surrounding environment.

Bacteria secrete enzymes to break down a wide variety of organic substrates (Chapman 2005) or secondary breakdown products. The level of decomposition will vary depending upon the amount of organic material available (Caple 2001).

Fungi usually are saprophytic organisms and are classified by their mode of reproduction, as saprophytes fungi obtain their nourishment from the degradation of dead organic matter. Most fungi are free-living including yeast, molds, and mushrooms. Most fungi are strict aerobes and can tolerate a low pH and a low nitrogen environment. Although fungi grow over a wide range of pH values (2–9), the optimum pH for most species of fungi is 5.6 to 6.5, and their nitrogen nutrient requirement for growth is approximately one-half as much as that for bacteria. Fungi have the ability to degrade cellulose, tolerate low nutrient levels, and grow in the presence of low moisture and low pH conditions (Gerardi, 2006).

2. MATERIALS AND METHODS

2.1 Sampling sites & methods

Samples were collected from forty two (42) different archeological objects at three different Jordanian Museums. The Museum of Jordanian Heritage, Faculty of archaeology and anthropology; Yarmouk University (YU), which contains different collections including: pottery, stone, wood, bone and others objects. The second Museum was Dar As-Saraya located on the southern side of Tallirbid (DS), and the third was the Museum of Archeology in Jordan University (JU).

Samples of the predominant alterations were taken from the surface of the objects. For non-invasive sampling, cotton swabs were pressed firmly over the surface of the object. Swabs were placed in sterile tube containing transport general purpose media (nutrient broth media (NB)) then transferred to the laboratory and incubated at 25°C and 30°C for 24 h. These cultures were performed to isolate the microorganisms present in the paint surface selected in order to determine the microbial population present in different areas with particular problems (color alteration, microbial growth, blistering, exfoliation, etc.). (Panel.1)

Panel 1. Different archeological objects (organic and inorganic in nature) were chosen and subjected to the current investigation. The indicated objects were sampled from Jordanian Museums Heritage, Yarmouk University (YU); Dar As-Saraya Museum (DS) and the archaeological Museumat the University of Jordan (JU).

3. MICROBIOLOGICAL METHODS

3.1 Culture based methods

3.1.1 Isolation and enumeration of microorganisms

Bacteria

Ten folds serial dilutions of collected samples were prepared and plated to enumerate colony forming units (CFU) on suitable media (nutrient agar (NA) and broth (NB)) (Difco, Detroit). Inoculated media was incubated at 30 ^{o}C for 24 h (Koren 2008). The colony forming unit per mL of nutrient agar (CFU/mL) was calculated for each sample.

Bacterial colonies with different morphology were picked and streaked again on fresh plates (one morphology per plate). There streaking process was repeated until morphologically consistent colonies were obtained for use in further studies (Wilson, 2010).

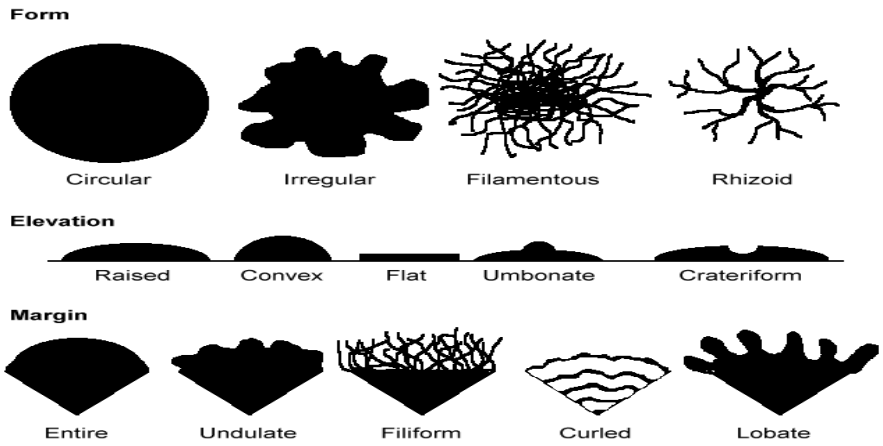
Fungi

Complete media (CM) was used to obtain pure cultures of fungi from the archeological objects taken from Museums. After 3 days of growth at 37 ^{o}C, fungal colonies were observed. Morphological identification of fungal isolates was performed microscopically.

3.2 Identification of bacterial isolates

3.2.1 Morphological characterization

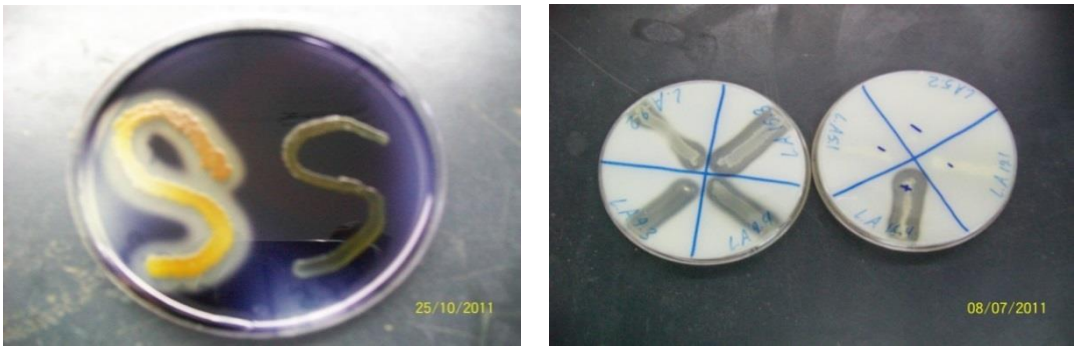
Morphological characterization of each bacterial isolate was performed according to color, size, colony characteristics (margin, form, and elevation) and gram staining. The identification of bacteria and fungi was based on how the organism grows on media and colonial morphology (shape, Size, Edge/ margin, Color and Elevation of colonies) (Holt 1994). (Panel. 2)



Panel 2. Morphological characterization.

3.2.2 Biochemical and physiological characterization

The bacterial isolates were further characterized using biochemical and physiological tests as described previously by Holt and his co-workers (1994).The purified isolates were identified as described in Bergey's Manual of Determinative Bacteriology (Holt 1994), Biochemical tests (34 tests) were used. In these tests, the bacteria were grown on the specific medium according to the standard preparation protocol with minor modification. The pH was adjusted according to the type of media used. *Escherichia coli* and *Bacillus* sp. were used as controls. (Panel 3).



A: Amylase enzyme test ***B: Casease enzyme test***

Panel 3 Amylase and Casease enzyme test.

3.3 Identification of fungal isolates.

Fungal isolates were grown at the permissive temperature (37^{o}C) on *Aspergillus* complete medium for 3-5 days, then identified microscopically (Laborlux 11, Germany), [Lens: x 100] (Figures 3, 4 and 5), (Fig. 4).

4. CONSERVATION PLAN

4.1 Integrated pest management (IPM)

Integrated Pest Management (IPM) is an effective and environmentally sensitive approach for pest management that relies on a combination of common-sense practices.

The care of collections and historic buildings involves many different disciplines, including conservation and management of both collections and buildings. The major factors causing deterioration are the environmental effects of humidity, light and temperature, and agents of decay such as insects, mould. All of these factors are inter-related and IPM seeks to resolve all pest problems using a holistic approach rather than reacting to each crisis as it arises. A well planned and executed IPM program will prevent problems or crises occurring and, in times of restricted budgets, will make much more effective use of limited human and cash resources. The reduced use of pesticides will lessen the risk of chemical damage to objects. (Pinniger 2004).

4.2 Preventive conservation

Preventive conservation is preferable to interceptive treatments, so ideally, objects should be kept in conditions that will minimize the danger of microbial colonies growth. This can be achieved through environmental control. Cool, dry conditions with good ventilation will generally prevent the growth of these species. Temperatures of 18 to 20°C and humidities of 50 to 55% RH, although lower humidity would further limit the potential for microbial growth (Mitchell 2010).

5. CONSERVATION WORK

Conservation of archeological objects was achieved by determining the method of treatment (that suite the object type) which would be used to stop or prevent microbial growth on archaeological objects.

5.1 Treatment with ultraviolet light (UV)

UV radiation kills bacteria by penetrating the organism outer layers, attacking DNA, RNA, and enzyme molecules, the primary mechanism is the formation of pyrimidine dimers and cytotoxic photoproducts that limit a cells ability to replicate and transcribe DNA. UV radiation at 265 nm is the most germicidal because this is the specific wavelength at which DNA maximally absorbs UV light (Yi Liu, 2010).

Bacterial and fungal species were treated by UV radiation (Typ: N-4K, Volt: 220, 254 nm). The treatment processes were applied on some archeological objects from the Museum of Jordanian Heritage-Yarmouk University. The treated objects include; Bone tools, bone (human skeleton), Snail shells and pendant in the shape of human face made of malachite stone. The treated objects (including organic and inorganic ones) were exposed to UV radiation in dark container for 5, 10, 15, 30 and 60 min.

5.2 Heat treatment

Heating is the most important and widely used method for sterilization of microorganisms; one must consider the type of heat, and most importantly, the time of application and temperature to ensure destruction of all microorganisms (Todar, 2009). The heating affect on the microorganisms by drying up the water contained in the cell. The role of this water to do chemical reaction inside cell, when exposed archaeological objects of heat inhibit chemical reaction in the microorganisms cell so killed this microbial. The treated objects were Bone (human skeleton), snail shells and basalt stone from the Museum of Jordanian Heritage-Yarmouk University. These objects were exposed to heat at 50 ^{o}C for 1 hr.

5.3 Chemical treatment (Thymol crystal)

Thymol is a white crystal with a distinctive aromatic odor. It is derived from thyme oil and may be mixed with camphor in its crystaline form.. No precise level for minimum exposure has been established. Thymol is sometimes used in its gaseous form (produced by heating the crystaline form to release thymol vapor) as a fumigant for small quantities of materials. In order to safely. handle treated objects following fumigation, materials must be aerated, preferably in a fume hood. This removes any residual protection against mold growth, but renders the materials safe for staff and patrons. Staff members working with items immediately after fumigation or in the area of the fumigation chamber should wear respirators approved for organic chemicals. Goggles and heavy weight, vapor barrier gloves should be worn when removing items from a chamber (Lee, 1989).

Bacterial and fungal species were treated by thymol crystal. The treatment processes were applied on archaeological textile from the Museum of Jordanian Heritage-Yarmouk University. The treated object is exposed to thymol crystal in close container for 1 week.

6. RESULTS and DISCUSSION

6.1 Isolation and enumeration of microorganisms -Enumeration of bacterial colonies from Museums

Results presented in (Fig.1) showed the number of bacterial colonies (CFU/mL) obtained from 17 out of 19 archeological objects subjected to the current investigation and preserved in the Museum of Jordanian Heritage, Yarmouk University. The obtained results indicate that the highest CFU values ($90x10^5$) were shown in samples taken from iron, whereas, the least values ($1.5x10^3$) were obtained from samples taken from the fabric of the bedouins tent. However, the remaining investigated objects have reflected CFU values within the range of $2x10^5$ to $40x10^5$.

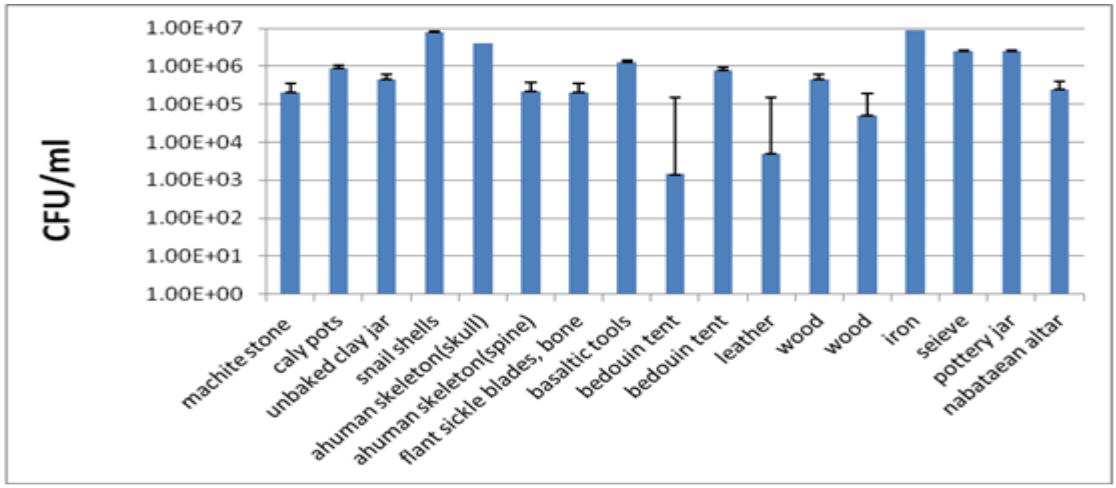


Figure 1 Viable cell count of bacterial isolates associated with the investigated archeological objects at the Museum of Jordanian Heritage, Yarmouk University (YU).

In addition, results presented in (Fig.2) showed the number of bacterial colonies (CFU/mL) obtained from 12 archeological objects subjected to the current investigation and preserved in the Museum of Dar as-saraya, Tallirbid (DS). The obtained results indicate that the highest CFU values ($300x10^4$) were shown in samples taken from Pottery jar, Bone tools and pendants, Mortar and grindstone and Fresco objects. However, CFU values in the range of $129x10^4$ to $300x10^3$ were obtained from swabbed samples collected from Pottery bowls and Bronze shackle, respectively. In contrast, the remaining tested objected showed CFU values in the range of $9x10^3$ to $107x10^3$, whereas, Scrapers and axes (Flint) have reflected no microbial growth.

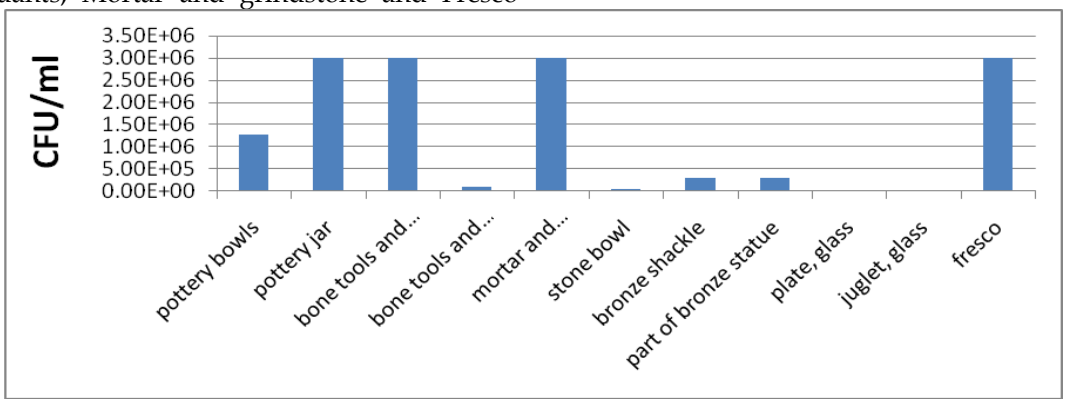


Figure 2 Viable cell count of bacteria associated with investigated archeological objects at the Museum of Dar as-saraya, Tallirbid (DS).

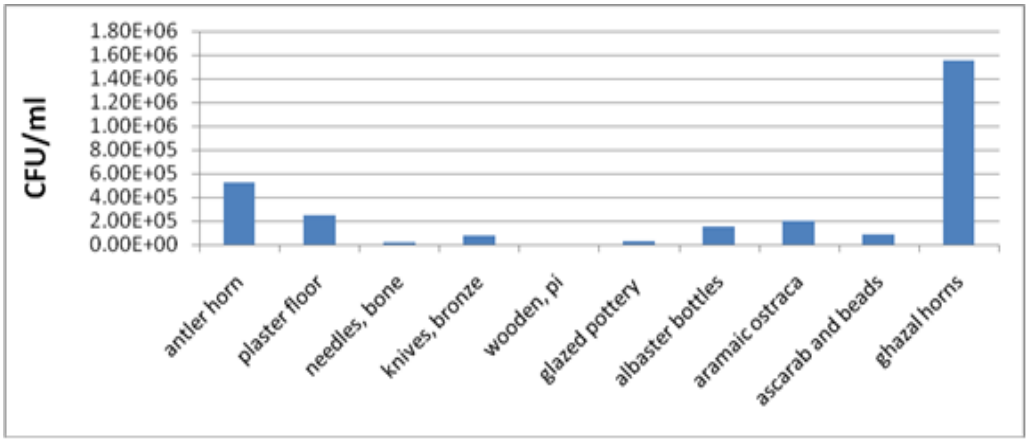


Figure 3 Viable cell count of bacteria associated with investigated archeological objects at the archaeological and heritage Museum, University of Jordan (JU).

Furthermore, results presented in (Figure.3) indicate that the number of bacterial colonies (CFU/mL) obtained from 10 archeological objects subjected to the current investigation and preserved in the archaeological and heritage Museum, University of Jordan. The obtained results indicate that the highest CFU values ($155x10^4$ and $53x10^4$) were shown in samples taken from Ghazal horns and Antler horn, respectively. The least CFU values ($30x10^2$) were seen with samples taken from wooden pieces. However, the remaining investigated samples have reflected CFU values within the range of $23x10^3$ to $254x10^3$.

6.2 Culture based analysis- Morphological characterization

Bacterial colonies were cultured on Luria agar (LA). The morphological description of bacterial isolates in the archaeological Museum at Yarmouk University The obtained morphology for bacterial colonies have ranged from circular, filamentous to punctiform, while diverse colony colors were obtained for isolates i.e. some were yellow, others have exhibited, creamy, milky white, or even orange color, indicating different pigments production by some bacterial isolates. On the other hand this might reflect the bacterial diversity among the isolates. Concerning the response of bacterial isolates to gram stain, indicated that the majority of bacterial isolates were gram positive rods, while some were gram negative rods and others were gram positive and negative cocci.

In addition, that were related to tested objects in the archaeological Museum at Yarmouk University,

indicate that 37 out of 56 bacterial isolates (66%) were found to be gram positive rods whereas, only 10 isolates (18%) were considered as gram negative rods. In addition, 8 isolates (14%) were classified as gram positive cocci while only one was found to be gram negative coccus (Fig. 4).

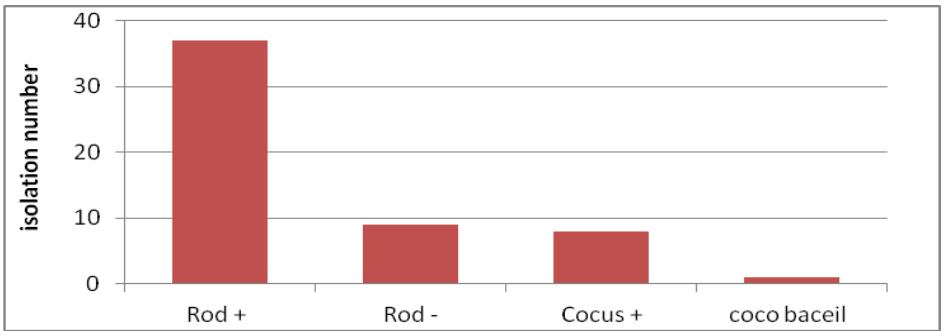


Figure 4 Diagram showed gram stain results for bacterial isolates obtained from archaeological objects at Yarmouk university Museum (YU).

Furthermore, the results in Dar as saraya Museum that 21 (72%) out of 29 bacterial isolates obtained from objects in were of the gram positive rods while, only one was of the gram negative rods. Also, 6 (21%) isolates were gram positive cocci and just one as a gram negative coccus (Fig. 5).

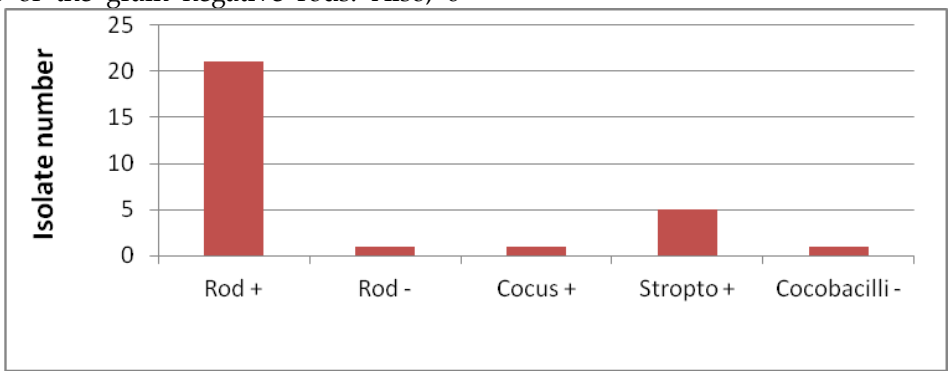


Figure 5 Diagram showed gram stain results for bacterial isolates obtained from archaeological objects at Dar As-saraya (DS).

Moreover, results showed that gram positive rods and gram positive cocci were represented by 17 (41%) for each type out of 41 bacterial isolates obtained from objects at the archaeological Museum, University of Jordan. Whereas, gram negative cocci and gram negative rods were represented by 6 (15%) and only one sample, respectively (Fig. 6).

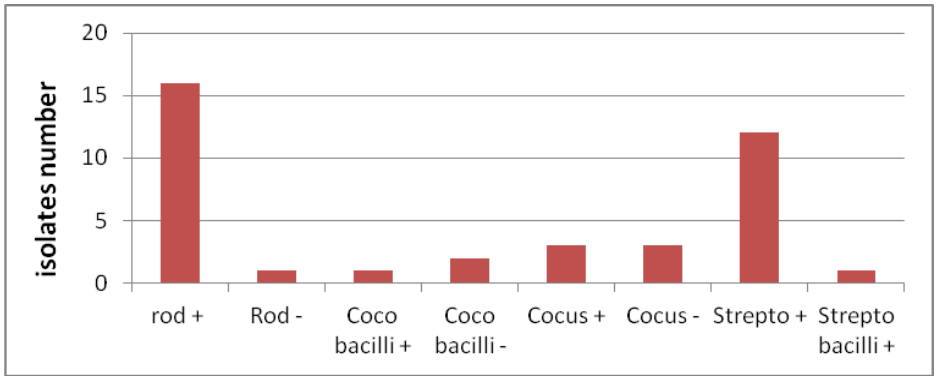


Figure. 6 Diagram showed gram stain results for bacterial isolates obtained from archaeological objects at the archaeological Museum, University of Jordan (JU).

6.3 Phenotypic characterization- Bacterial isolates from Museums

A total of 96 bacterial isolates were obtained from the three investigated Museums. However, 35 of these isolates were obtained from different types of objects including malachite stone, Clay pots, bone, Unbaked clay jar, Snail shells, Flint, basalt stone, Fabric, **leather**, wood, iron and lime stone at the Jordanian Heritage Museum-Yarmouk University.

While 25 isolates were isolated from the Museum of Dar as-saraya , Tallirbid. These isolates inhabited pottery, **bone**, flint, mortar, bronze, glass and fresco objects. Also, 36 isolates were found to inhabit various types of archaeological objects (horn, plaster, bone, bronze, wood, glazed pottery and Alabaster) at the University of Jordan Museum. All of the above mentioned samples were identified by biochemical tests identification diagrams.

Results presented in the archaeological Museum, Yarmouk University indicate that 19 archaeological objects were subjected to the current investigation at, where these objects were found to be contaminated by 11 bacterial and 9 fungal species. However, of these 19 objects 5 were found to be inhabited by the fungus *Penicillium chrysogenum*, 7 objects inhabited by *Aspergillus niger* (the black mould) and 8 objects were contaminated by yeast. The fungus *P. chrysogenum* was found to inhabit mainly bones, wooden objects and clay pots. Yeast was found in bones, snail shells, wood iron and fabrics. However, *A. niger* was contaminating bones, wooden objects, clay pots, unbaked clay jars and basalt.

Furthermore, results in the archaeological Museum, Yarmouk University indicate that 7 objects out of the 19 tested were contaminated by *Corynebacterium spp* while 6 were contaminated with *C. aquatium*. Results indicate that of the organic objects that are preserved inside show case snail shells were contaminated by *Salmonella spp* and yeast, whereas, human skeleton was contaminated with *corynebacterium spp, Staphylloococcus spp*, *E. coli* and *Aspergillus niger* as a fungus.

However, the organics outside the case were contaminated by *corynebacterium, C. aquatium, A. niger, A. nidulans, P. chrysogenum* and yeast. Furthermore, the inorganic objects inside the show case were mainly contaminated by *Corynebacterium spp, C. aquatium, Staphylococcus aereus* and *E. coli*. Also, the following fungal species were detected: *A. niger, P. italicum, P. digitatum, A. nidulans* and *P. chrysogenum*. Moreover, the inorganics in the outside show cases were found to be mainly inhabited by *Pseudomonus spp, Staphyllococcuc aereus* and *E. coli*. In addition, the following fungal species were found in such objects: yeast, *P. italicum*, A. niger, *A. nidulans* and *P. chrysogenum.*

Results presented at Dar as saraya Museum indicate that 12 archaeological objects were subjected to the current study, where these objects were found to be contaminated by 8 bacterial and 4 fungal species. However, of these 12 objects 5 were found to be inhabited by yeast, 1 object was inhabited by *P. notatum* and 1 by *P. digitatum*. The yeast fungus was found to inhabit mainly bones, glass and fresco objects.

Furthermore, results indicate at Dar as saraya Museum that 7 objects out of the 12 tested were contaminated by *C. pseudodiphtheriticum* while 4 were contaminated with *C. aquatium*. The *C. pseudodiphtheriticum* was found on bones, pottery, stones, bronze and glass objects, whereas, *C. aquatium* inhabited mortar, stones, bronze and glass. Results indicate that of the organic objects that are preserved inside show case bones were contaminated with *B. cereus, C. pseudodiphthrticum* and *yeast*, whereas, the inorganic objects inside the show case were mainly contaminated by *C. pseudodiphthriticum, Clostridium sp*, *C. aquatium*, and *yeast*. These mentioned microbes

were found to inhabit pottery, glass, flint, stones and bronze objects.

Results at the archaeological Museum, University of Jordan indicate that 11 archaeological objects were subjected to the current investigation, where these objects were found to be contaminated by 10 bacterial and only one fungal species which is yeast. However, of these 11 objects 9 were found to be inhabited by the yeast fungus and these p objects were represented by bones, antler horn, wood, horns, plaster floor, bronze, glazed pottery.

In addition, 6 objects representing antler horn, wood, glazed pottery and bone were contaminated by *C. aquatium*, whereas, 4 objects (bone, horns, wood and alabaster) were contaminated with *Corynebacterium pyogenes*. Furthermore, 7 objects were contaminated with *Listeria monocytogenes* and these 7 objects include wood, plaster floor, pottery, alabaster and glazed pottery. Results indicate that of the organic objects that are preserved inside show case the antler horn was found to be contaminated with *Micrococcus* spp, *C. aquatium* and yeast, whereas, the horn was contaminated with *Corynebacterium* spp, *B. cereus* and yeast as a fungus. In addition, bones were contaminated by *B. subtilis*, *B. cereus*, *Corynebacterium* spp and yeast. However, the inorganic objects were contaminated by various bacterial species and yeast.

6.4 Conservation work

The treated objects (including organic and inorganic ones) were exposed to UV radiation for 15, 30 and 60 min. However, the 15min period was effective in reaching a microbial killing rate. (Abdel-Kareem 2010)

Heat treatment at 60°C for 1 hr, was proven to be ineffective in treating the indicated archaeological objects, because, bacterial as well as fungal growth was achieved after the treatment (Table 1). In addition, the chemical treatment was shown to be effective in controlling microbial growth, because bacterial growth was completely eliminated after treatment (Table 2).

Table 1. The treatment of various archaeological objects with UV radiation and heating.

Samples	UV radiation/254nm			Heating/50°C
	Time			
	15Min	30Min	60Min	60Min
Bone tools	No Growth	No Growth	No Growth	Not treated
Bone (human skeleton)	No Growth	No Growth	No Growth	Growth of bacteria and fungi
Snail shells	No Growth	No Growth	No Growth	Growth of bacteria and fungi
Basalt stone	Not treated	Not treated	Not treated	Growth of bacteria and fungi
Pendant in the shape of human face made of malachite stone.	No Growth	No Growth	No Growth	Not treated

Table 2. The treatment of various archaeological objects with UV radiation and thymol crystals.

Samples	UV radiation/ 254nm			Before thymol crystal	After thymol crystal
	Time				
	Before UV	After 5Min UV	After 10Min UV		
Textile	Not treated	Not treated	Not treated	Abundant Bacterial growth / no fungal growth	No Growth
Bone A	No Growth	No Growth	No Growth	Not treated	Not treated
Bone B	Abundant Bacterial and Yeast growth	Bacterial growth (3-4 bacterial colonies) and abundant Yeast growth	Bacterial growth (3-4 bacterial colonies) and abundant Yeast growth	Not treated	Not treated

7. CONCLUSION

We concluded that microorganisms (bacteria and fungi) responsible for biodeterioration of archeological objects in three Jordanian Museums (The Museum of Jordanian heritage- Faculty of Archaeology and Anthropology at Yarmouk University, Dar assaraya Museum in tallirbid, and Museum of Archeology- Jordan University). Results indicated the presence of 8 fungal species on tested archeological objects which includes Aspergillus niger,

Aspergillus nudulans, Aspergillus fumigates, Penicillium chriyso-genum, Penicillium digitatum, Penicillium italicum, pithium sp and yeast. On the other hand, the identified bacterial isolates belongs to 11 bacterial species which includes *Micrococcus spp, Corynebacterium aquatium, B. subtilis, B. cereus, Listeria monocytogenes, Corynebacterium pyog-enes, Salmonella. sp, E. coli, p. pseudoaclingenes, Staphylococ-cus aureus, and C. pseudodiph-theriticum*. Most of organic archeological objects showed the highest microbial growth (bacteria and fungi). Treating objects including: organic and inorganic ones with UV radiation for 15 min, was effective in reaching a microbial killing rate, also chemical treatment (Thymol crystals) was effective. But heating treatment was proven to be ineffective in treating archae-ological objects where, bacterial as well as fungal growth was achieved after heat exposure at 50 ^{o}C for 1 hr.

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