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Characterization of Microbiota Deteriorating Specific Coptic Manuscripts, Coptic Museum, Egypt

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Abstract: *Microbiota colonizing manuscripts (flax, parchment and leather binding) within the Coptic Museum, Cairo, are bacteria (Staphyloccus aureus; Bacillus pumilus; Bacillus subtilis; Bacillus firmus; Pseudomonas sp., Micrococcus sp.), fungi (Penicillium sp., Aspergillus niger, Aspergillus terrus, Aspergillus flavus, Acremonium vitis, Botrytis cenera., Fusarium sp., Geotrichum spp., Mucor spp, Stachyliduim spp., Stachybotrys chartarum and Trichoderma spp.).*

The investigated manuscripts were stained with yellow and red stains. The isolated microorganisms produced pigments on synthetic media, and FTIR spectra of these produced pigments proved that are of carotenoid. Moreover, the isolated fungi and bacteria are cellulease and collagenase enzyme producers; these enzymes could decompose carboxy methyl cellulose (CMC) into short chains of free mono sugars and decompose collagen (animal glue) into free amino acids and ammonia as end product.

Keywords: *Aesthical damage; Carotenoid, Collagenase enzyme; Coptic manuscripts, Melanin, TiO_2 Nano particles.*

1. INTRODUCTION

Manuscripts are store for knowledge that needs to be saved. In Egypt, Coptic manuscripts were subjected to plunder and deterioration movement, so from the 17^{th} century onwards, local authorities allowed the Copts to renovate old churches also, old manuscripts were being copied and new ones were created after the destroying and burning of old icons movements and manuscripts prevailed in 14^{th} -15^{th} centuries according to the old Christian religious and artistic traditions (Sakr et al., 2016).

Most manuscripts assigned to this period were made either of flax or parchment with leather book bindings, and parchment was widely used as writing support from the 2^{nd} century B.C. till the end of middle age (Florian, 2007), and in the 18^{th} century AD, it became one of the most common writing supports were used to renovate the old destroyed manuscripts (Woods, 2006).

Library documents are generally composites of different materials (flax, parchment and leather book binding); each with different possible responses to environmental changes (Mesquita et al., 2009). Under unsuitable storage conditions, these manuscripts are subject to microbial deterioration.

Microorganisms of fungi (*Cladosporium cladosporioides*, *Davidiella tassiana*, *Alternaria alternate*, *Eurotium appendiculatum*, *Aspergillus proliferans*, *Acremonium polychromum*, *Penicillium citrinum*) and bacteria (*Bacillus* and *Staphylococcus*) are involved significantly in deterioration of parchment, book bindings, papyrus and paper documents through staining of colonized cultural objects with irreversible degradation black spots colors, or carotenoid with red, orange and yellow color, foxing colonized manuscripts and hidden decorations and wording (Sterflinger & Piñar, 2013; Gutarowska et al., 2012; Karbowska-Berent et al., 2011). These bio pigments are diffused into and within fabric of colonized objects resulting in significant loss in value and quality of colonized materials (Gutarowska et al., 2016; Borrego & Perdomo, 2015). These stains are irreversible and resistant to chemical, physical and biological disintegration for long period even after microbial colonies are controlled (Pinzari et al., 2011).

The other biodeterioration aspect ascribed to microbial colonizing of manuscripts and other archival material is the structural damage by secretion a wide range of enzymes in particular collagenase and cellulease enzymes that could decompose complex cellulose and collagen based cultural heritage objects such as books and other paper documents into short chain of free mono sugars and amino acids respectively soluble in water that could be used carbon source by colonizing microorganisms for their growth and colonization (Cybulska et al., 2008) thus reducing mechanical properties of colonized objects, and in the advanced phases of deterioration these objects may turn into powdery form (Niesler et al., 2010).

Because of harmful effect of colonizing microorganisms, and obstacles imposed by the traditional methods in decontaminating microbial micro biota such as biocides and antibiotics (Rai et al., 2009), the new trends are using green and eco-friendly technologies in decontamination of microorganisms, such as gamma irradiation (Abdel Haleim et al., 2013) and DBD plasma (Sakr et al., 2015), and recently, application of NPs in decontamination of microorganisms deteriorating cultural heritage objects has received great attention (Fierascu, 2013).

Nanoparticles are of great interest that may be assigned to their multiple potential applications (Knetsch and Koole, 2011). NPs have unique physicochemical properties including ultra small size, large surface to mass ratio, a distinctive reactivity with biological systems, and could be used in combination with physical techniques such as DBD plasma and gamma irradiation or with chemical such as antibiotics (Zhang et al., 2011).

Application of nanoparticles in decontaminating microorganisms colonizing cultural heritage objects is still in its infancy with some exceptions such as using Titanium oxide (TiO_2) nano particles are antimicrobial agents against both fungi (*Fusarium oxysporum*, *Rhizopus stolonifer* and *Aspergillus flavus*) and bacteria (*Staphylococcus warnei* and *Micrococcus luteus*) isolated from mural paintings within royal tombs (Tausert and Setnkht, Seti I, Ramsis V, Ramsis VI) at Valley Kings, dated back to the New Kingdom, and found that the optimum concentration is 160µg gave an inhibition zone ranged from 11-14 mm. The inhibition effect of TiO_2 is a function with time, since it has been reported that TiO_2 treatments had significant inhibitory effect on the growth of microbes during 24 and 72 hs of incubation (Khalaphalla & El-Derby, 2015).

The lethal effect of nanoparticles against colonizing microorganisms is attributed to easily reaction of silver nanoparticles with cell membranes and releasing free radicals (Okafor et al., 2013), and these free radicals can attack membrane lipids causing dysfunctions microbial cell membrane (Soo-Hwan et al., 2011).

The aim of this paper is to identify the putative causal agents and to suggest a model of biodeterioration and clarify the damage done by biodeteriogens to the structure of parchment collagen and flax paper, and evaluate the antibacterial activity of some nano particles against the isolated bacteria.

2. Materials and Methods

2.1. Microbial Sample Collection

Twenty three microbial samples were taken from Coptic manuscripts of flax and parchment and leather book bindings dated back to 17^{th} century are housed within Coptic Museum, Old Cairo (**Fig**.1) suffering from different deterioration symptoms such as microbial stains greenish in the manuscript no. 1679 (**Fig.3**), grey color stains in manuscript no.64 (**Fig. 4**), and red and orange microbial stains in the manuscript no. 759 (**Fig**. **5a**). In addition to microbial deterioration, the investigated manuscripts are subjected to other deteriorations symptoms such as dissolving inks in the manuscript no. 759 (**Fig**. **5b**). Isolation and biodeterioration symptoms are illustrated in Table 1.

Figure1. *Location of Coptic Museum where the investigated manuscripts are housed*

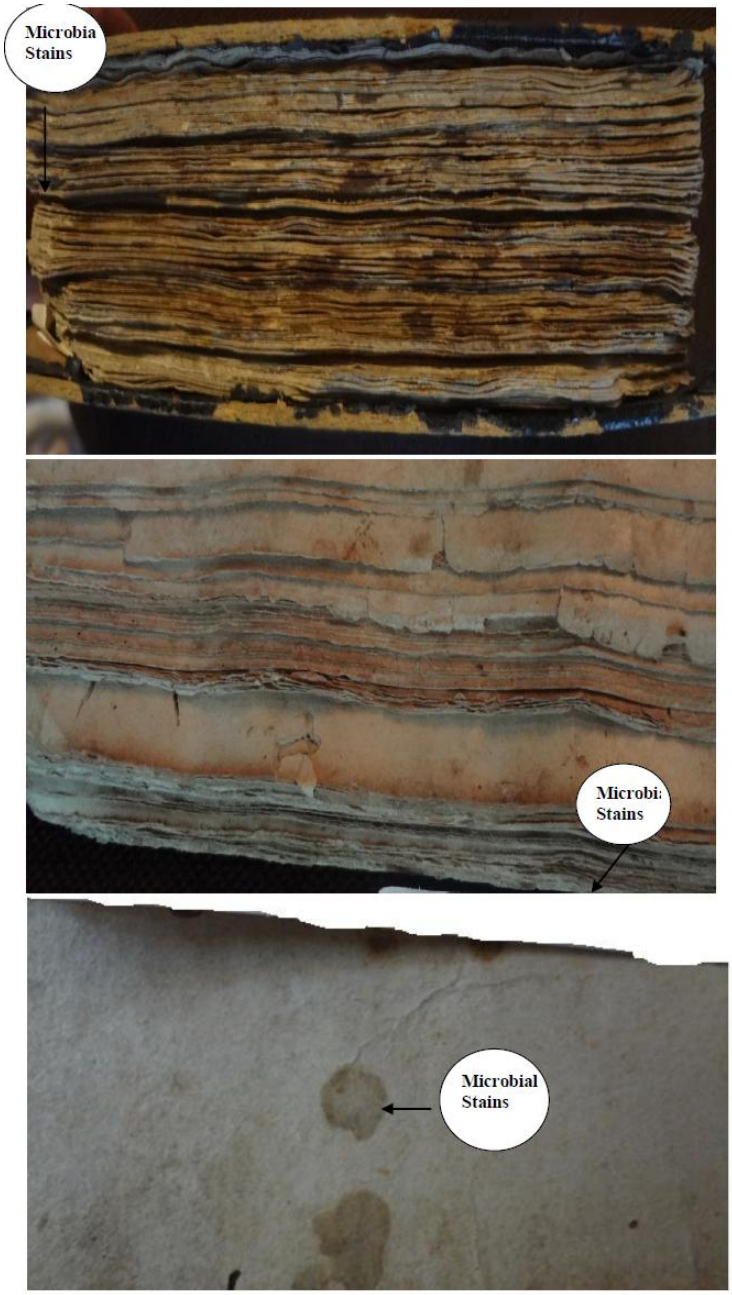


Figure2. *Disfiguration of Coptic manuscripts by microorganisms (a) no. 863.1 (b) no. 5238, (c) no. 692*

Figure3. ***(a)*** *Disfiguration of Coptic manuscript no. 1679, 18th AD century by olivey green pigment produced by microorganisms. (b) Microbial stains on the book binding no. 1352*

Figure4. *Disfiguration of Coptic manuscript no.64 made parchment by microbial colonization*

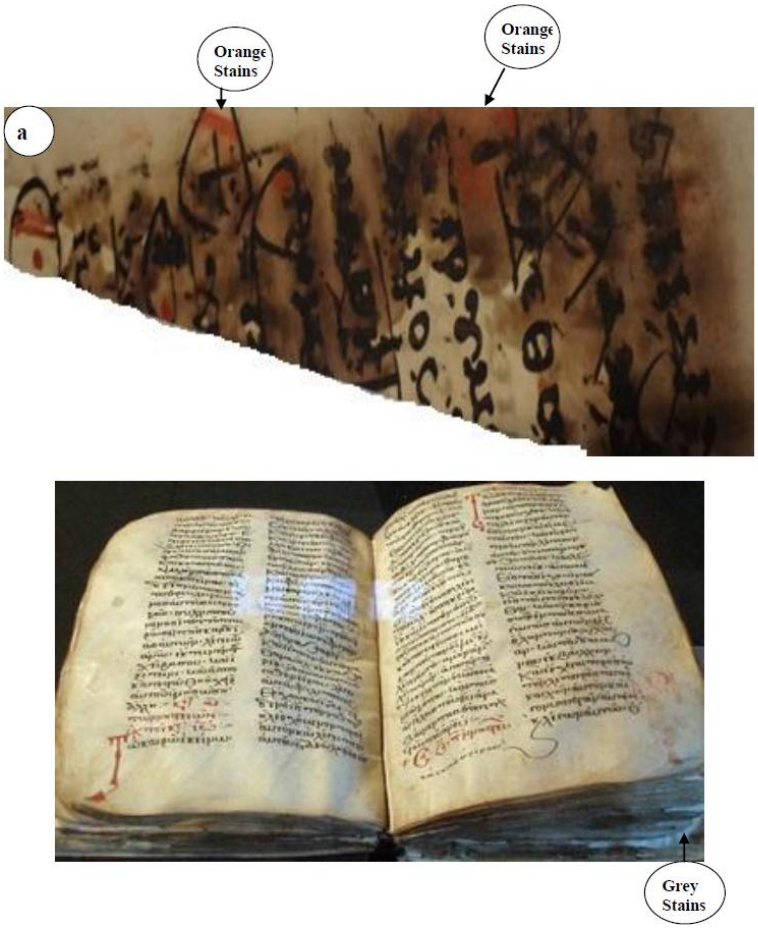
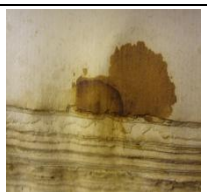


Fig5. *(**a**) Staining with orange color (manuscripts (no. 759) (**b**); Dissolving inks of Coptic manuscripts*

Table. *Location of samples within Coptic museum (CM)*

Sample number	object	Object number in CM	Date	observation	Photo
1-2	Manuscript (Flax)	1679	18^{th} century	Brown stains, stains with petroleum color *Bacillus subtilis*	
3	Manuscript (Flax)	692	-	Grey stains	
4	Manuscript (Flax)	759	1428 shohada (viz…..)	Dissolving inks	

5	Manuscript (Flax)	2665	-	Dissolving inks	
6	Manuscript (Flax)	5250	-	Dissolving inks	
7-8	Manuscript (Flax)	5238	1349 shohada		
9	Manuscript (parchment) Actinomycetes (6 colonies) and bacteria (6 colonies) are the most present produced pale brown pigment	64	-	stains	
10	Manuscript (Flax)	114	1500 shohada		
11	Manuscript (Flax)	772	1560 shohada	Black stains with wax	
12	Book binding (leather)	4181			
13	Book binding (leather)	5242	1380 shohada	With brown color , rupture	
14	Book binding (leather)	1352			
15	Stains on paper	1020	1168 shohada		

16	Book binding (leather)	1007	1421 Shohada		
17	Book binding (leather)	1004			
18-19	Spots on brown color (Flax)	723	1466 Shohada		
20	Manuscript (parchment)	863.3	1450 Shohada		
21	Manuscript (parchment)	863.1			
22	Manuscript (Flax)	1026	-	Wax spots	

2.2. Isolation of Microbial Isolates

Microbial isolates are obtained using sterile cotton swabs according to Pinzari et al., (2011) where sterile cotton swabs are wiped across spots showing visible damage, transferred to the Lab. in sterile test tubes.

In Microbiology Labs., cotton swabs were soaked in 5 ml saline (0.85% NaCl) and vortexed for 10 mins using programmable rotator mixer to release the entire microbial load according to Niesler et al., (2010), then cultured onto an appropriate media.

Fungal isolates were cultured onto Dox-Czapek plates (g/l) (30 sucrose, K_2HPO_4 1, $NaNO_3$ 3, $MgSO_4.7H_2O$ 0.5, KCl 0.5, $FeSO_4.5H_2O$ 0.01, agar 20 in 1000 ml distiled water), incubated for 7 days at 28 °C until single colony appeared. Single colonies were identified morphologically according to the identification keys of Booth (1977); Raper & Fennell (1977); Raper et al., (1968).

But bacterial isolates were cultured onto nutrient agar paltes (5g peptone, 3g beef extract, NaCl 5g, agar 20 g in L distilled water, pH 7-7.1), incubated for 72 hs. 24 at 28°C for fungi and bacteria respectively to obtain colonies with mature fruiting bodies or reproductive structures. All microbial isolates were purified twice till single colony was appeared, and the purified isolates were used for further investigations.

All Bacterial isolates were identified biochemically using M ALDi-TOF-MS (Matrix assisted Laser desorption ionization Time of flight mass spectrometry).

2.3. Bio –Pigments Investigations

To determine the nature of produced bio-pigments, the extracellular bio pigment produced by identified microorganisms, in particular *Fusarium* oxysporum was extracted and purified according to

Sterflinger et al., (1999) whereas Erlenmeyer (250-mL) flasks were used. Each flask contained 50 mL of the Nutrient and Dox broth medium for bacteria and fungi respectively. Each flask was inculcated with identified bacteria and fungi in both shaking and static condition at 28 °C for 2, 7, 21, 30 days.

Broth was centrifuged at 3000 rpm for 5 mins., the biopigments in broth medium were extracted on thin layer chromatography (TLC) on silica gel plates (60 Merck, Damstadt, Germany) using a solvent mixture of *n*-hexan and acetone 92:8 *v*/*v* (Sakr et al., 2012) and the extracted biopigments were analyzed using FTIR Spectroscopy (JASCO FT-IR 61000, National research Centre, Cairo). Functional groups resulted in FT-IR spectra were interpreted according to (Derrick et al. 1999).

To test sensitivity of the produced pigment to pH, two test tubes were used, each one contained 1 ml of supernatant, and 1 ml of 5% NaOH and H_2SO_4 were dropped in each tube, and the resulted color was observed.

2.4. Determination of Cellulease Enzyme Activity

The analysis of the relationship between the spoiling microorganisms and the substrates can be helpful in documenting the symptoms of the degradative attack on the different components of the cultural material. To determine cellulease enzyme activity of identified microbial isolates, *Bacillus subtilis* the most common isolated was cultured on nutrient agar supplemented with 2% carboxy methyl cellulose (CMC) as sole carbon source and inhibition zone was estimated in mm.

In addition, to confirm the enzymatic activity *Bacillus subtilis* 250 ml flasks were used, Each flask contained 100 ml of broth medium (pH was adjusted to 7) supplemented with 2% CMC, inoculated with 10% spore suspension (1×10^6 spores / ml) and incubated at 28 °C for bacteria and fungi. At the end of incubation period, the biomass was filtered off and the filtrate was cleared by centrifugation at 3000 rpm for 15 min. Free mono sugars in the media resulted in enzymatic decomposition of CMC were determined using DNS method (Dinirto salicylic acid $[O_2N)_2\ C_6H_2$-2-$(OH)CO_2H]$, and red color modified according to Niesler et al., (2010).

2.5. Determination of Collagenase Enzyme

Collagenolytic activity of isolated microorganisms was determined according to Guiamet et al., (2010) where bacteria and fungi were cultured onto to nutrient broth, starch-nitrite broth and Dox broth respectively, animal glue was used as carbon source, and incubated for one week and one month for bacteria and fungi respectively. Supernatant was cleared by centrifugation for 5 mins. and 3000 rpm and amino acids were determined using high performance liquid chromatography (HPLC) amino acid analyzer LC300 Eppendorf Germany (National Research Centre, Dokky, Giza.

2.6. Determination of Antimicrobial Activity of Nano Particles

To determine the antimicrobial activity of nano particles of TiO_2, CaOH, carbon (C) against identified bacteria ((*Staphyloccus aureus*; *Bacillus pumilus*; *Bacillus subtilis*; *Bacillus firmus*; *Streptococcus* sp.; *Pseudomonas* sp., *Micrococcus* sp.), where nano particles in concentration 100 ug in DMSO (dimethyl sulfoxide) using filter paper discs methods. Efficacy of nano particles was estimated by inhibition zone in mm.

3. RESULTS

3.1. Identification of Microbial Isolates

Twenty one isolates pointed out that bacterial isolates are belonging to *Bacillus subtilis*, *B. pumilus*, *B. firmus*, and *Staphyloccus aureus* with a predominance of spore-forming bacteria. *Staphyloccus aureus* was commonly isolated from parchment (6×10^3 cfu) in pure form (**Fig**. 7).

On the other hand, morphological identification isolated fungi are belonging to the following genera: *Aspergillus* (*Aspergillus* spp., *A. niger*, *A. terrus*, *A. flavus*, *A. carbonarus*), *Acremonium vitis*, *Botrytis* spp., *Fusarium* sp., *Geotrichum* spp., *Penicillium* sp., *Stachybotrys cenera*, *Stachyliduim* spp.

3.2. Identification of Bio Pigment

Morphologically, investigated manuscripts were stained with different colors, and *Staphyloccus aureus* was isolated from yellow stained parchment (object no. 863.1) which produced yellow or gold color on the synthesized media (**Fig**.6). In addition, *Fusarium oxysporum* produced a pink pigment that diffused into synthetic media (**Fig**. 7c).

Furthermore, FTIR spectra of red pigment gave a strong band at 3457 cm^{-1} characterizing quenon group (O_2-N-O-R), so the carotenoid pigment ($C_{40}H_{50}$) (**Unpublished data**), so carotenoid pigment is the most probable**. It has been found** that the production of pigment was increased with the age of incubation, and this biopigment was non pH sensitive in alkaline media, no color change was observed with neither alkalinity nor acidity.

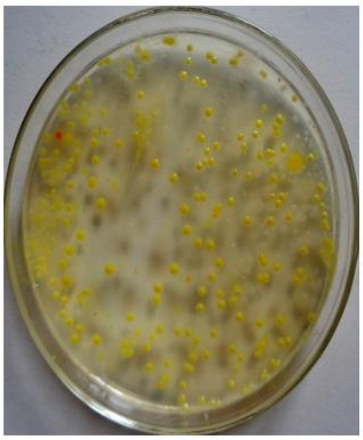


Figure6. *Bacteria with yellow color isolated from object no. 863.1 parchment, clear zone on gelatin substrate*

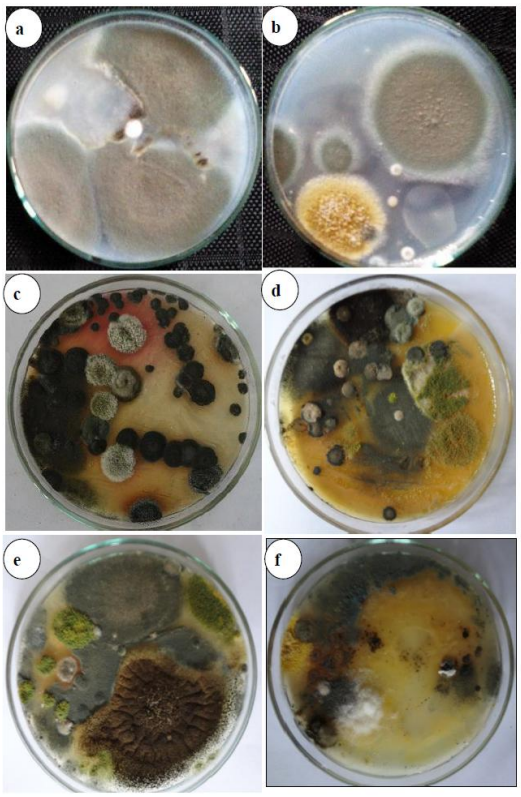


Figure7. ***(a)*** *Laboratory cultures illustrate dominance of Aspergillus flavus in deteriorated manuscripts with olivey green stains.* ***(b)*** *Association between Aspergillus flavus, Aspergillus terrus and Fusarium sp.,* ***(c)*** *Aspergillus niger with black color and Fusarium oxyspoum* ***(d)****. Microbiota colonizing flax manuscripts and parchment.* ***(e)*** *Aspergillus flavus* ***(f)*** *Mucor sp*

3.3. Enzymatic Activity of Microbial Isolates

Identifies fungal isolates showed enzymatic activity no. 1679 made from flax cultured onto CMC plates showed enzymatic activity in from of clear zone approximately 2.5 and 3.5 cm (**Fig**.8), but bacterial and *Streptomyces* isolates showed moderate cellulease enzyme activity.

Current results pointed out that *Aspergillus flavus*, *Penicilluim* sp., and *Aspergillus terrus* have higher growth rate on Na-CMC, while *Fusarium* sp. has moderate growth. With regard to bacterial cellulease enzymatic activity, it was found that *Bacillus pumilus* and *Bacillus firmus* have higher cellulease enzyme activity, while *Bacillus subtilis* has moderate activity and *Staphyloccus aureus* has lower activity.

In enzymatic assay, bacterial isolates showed growth on the CMC-Na, and gave a red color measured spectrophotometrically at 240 nm, and variety in their enzymatic activity.

Our findings pointed out that *Staphyloccus aureus* golden color on plates and *Bacillus subtilis* have higher growth on animal glue as substrate and they were commonly isolated from parchment and book binding objects.

On the other hand, *Penicilluim* sp. and *Aspergillus niger* are the most present of animal glue as a substrate on both broth and plates.

Antimicrobial activity of nano particles pointed out that neither carbon nano particles nor calcium carbonate nano particles has inhibitory effect at all on the identified microorganisms, but titanium oxide (TiO_2) was the exception with inhibition zone ranged from 9-19 mm. **Fig**.9 pointed out inhibition zone 19 mm with *Bacillus pumilus*, 15 mm with isolate *Staphyloccus aureus* 13 mm with *Bacillus firmus* 9 mm with *Bacillus subtilis*, 10 mm with *Bacillus subtilis* 7 mm with *Micrococcus* sp.

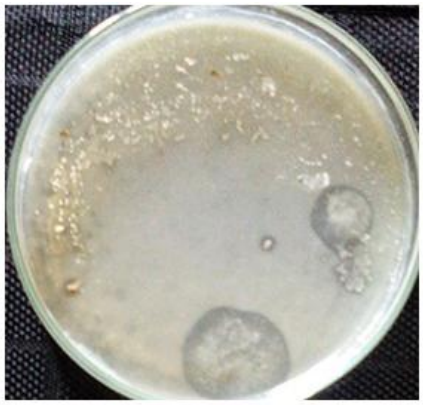


Figure8. *Cellulease activity of Enzymatic of Bacillus subtilis on CMC as substrate*

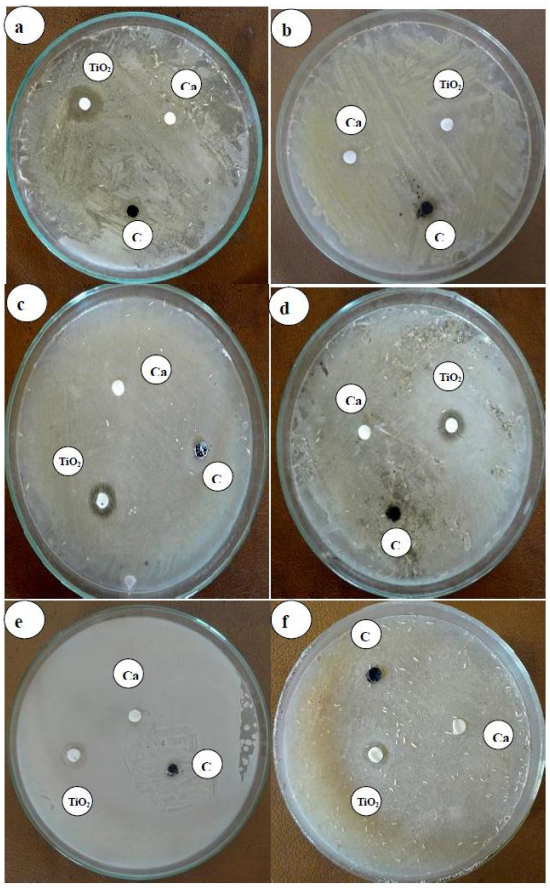


Figure9. *Effect of TiO_2 nanoparticle sizes and inhibition zone (a) Bacillus pumilus (b) Staphyloccus aureus (c) Bacillus firmus (d) Pseudomonas sp., (e) Bacillus subtilis (f) Micrococcus sp*

4. DISCUSSION

Morphological and biochemical identification revealed that bacterial isolates are members of the phylum *Bacillus* that are belonging to (*B. subtilis*, *B. firmus*, *B. pumilus*), and were the most dominant in the microbiota isolated from deteriorated parchment and archival materials. This may be attributed to the ability of *Bacillus* to produce a wide range of antibiotics such as subtilosin, surfactin, bacilysin, amicoumacin, lantibiotics subtilin, ericin and mersacidin could inhibit or at least inactivate the competitive microorganisms of fungi and other bacterial genera (Stein, 2005).

The other deterioration symptoms caused by *Bacillus subtilis* is decomposition of animal based fibers in parchment **through the** enzymatic **pathway which turned** collagen substrate turned into liquid or semi liquid (Nugari, 2005).

Our data pointed out that *Staphyloccus aureus* commonly isolated from stained parchment and book binding (samples no. 9, 13, 20, 21) rather than flax manuscripts and involved significantly in deterioration of parchment, (Kraková et al. 2012) that may be ascribed to the matter of fact that *Staphyloccus aureus* is opportunistic in nature and its higher adaptability to different adverse environmental conditions, its nearly pure colony on the synthetic media confirm that its presence not airborne (Abrusci et al., 2005).

Furthermore, *Staphylococcus aureus*, inter alia (*Aspergillus niger*, *Penicillium*, *Alternaria*, *Bacillus*, *Staphylococcus*, *Micrococcus* sp., *Mucor*, *Chaetomium*, and *Streptomyces*) were the most potent biodeterogens colonizing vegetable tanned parchment through the enzymatic hydrolysis causing both aesthical and structural damage (Strzelczyk & Karbowska, 1994).

Our results pointed out that fungal isolates obtained from both flax manuscripts and parchment are belonging to *Acremonium vitis*, *Aspergillus flavus*, *Aspergillus terrus*, *Aspergillus carbonarus*, *Botrytis* sp., *Fusarium* sp., *Geotrichium* sp., *Mucor* sp., *Penicillium* sp., *Stachyliduim* spp., *Trichoderma* sp. The involvement of fungi in deterioration of library and archival materials was put onto the evidence, it has been referenced that *Aspergillus* and *Penicillium* sp., are considered the primary and the most potent colonizers of organic cultural heritage objects, that may be ascribed to their saprophytic lifestyle and adopting various lifestyles (Brusci et al., 2005). In general, *Cladosporium, Aspergillus* and *Penicillium* phyla have been described as archive materials and indoor air contaminants (Sterflinger, 2010).

Visually, manuscript no. 1679 is stained with olive or greenish stains where *Aspergillus flavus* was isolated, this in agreement with Ettenauer et al., (2014) reported that most fungi isolated from deteriorated paper and parchment caused foxing the colonized cultural heritage objects with different colors, in particular olivey and black colors.

Our results showed variety of microorganisms that may be ascribed to two main determinants, the first one is storage conditions, it has been reported that *Aspergillus niger*, *Penicillium sp.*, *Cladosporium harbarum*, *Bacillus subtilis* are most present in foxed archival materials, papers and documents stored in boxes with bad ventilation (Valentin, 2010). The second one is bioreceptivity of paper and parchment to microbial colonization due to its hygroscopicity and composition of colonized objects (cellulose, hemi cellulose, collagen and adhesives) represent an abundant carbon source for hertrotrophic colonizers (Sequeira et al. 2012).

Current results pointed out that *Stachybotrys chartarum* was isolated from manuscript no. Manuscript of flax. This in agreement with Hagaggi and Salah, (2016) stated that *Stachybotrys chartarum* and *Aspergillus flavus* were isolated from deteriorated papers and documents.

FT-IR spectra of red pigment produced by microbial colonization on objects nos. 64, 4181, 5242, 1352, 863.3, 863.1 gave an intense band at 3457 cm^{-1}, the fingerprint region of quinonoxime (O_2-N-O-R) (Avram & Mateescu, 1990) so carotenoid pigment is the most probable. Carotenoid series have colors rang from yellow, orange, red, pink and violet (Sakr et al., 2012), mainly composed of three series are β carotene ($C_{40}H_{56}$), γ carotene ($C_{40}H_{56}$) and rhodoxanthin ($C_{40}H_{54}O_2$), and this pigment involved in patination of rock surfaces (Sterflinger et al., 1999) and paper manuscripts with brightly colored patinas (Pinzari et al., 2011) in form of foxing due to accumulation of pigments diffused in and within collagen and flax fibers in the colonized manuscripts (Mesquita et al., 2009).

Moreover, the morphological observation pointed out that the microbial alterations detected on under investigation parchment manuscripts have the following characteristics: red, orange or purple maculae, with anucleated peripheral halo, isolate or coalescent, this result in agreement with Pinzari et al., (2012) reported that deterioration aspects are common on parchment manuscripts.

In this context, Gutarowska et al., (2016); Pasquariello et al., (2005) reported that groups of pigment-producing bacteria include *Achromobacter* sp., *Bacillus* sp., *Brevibacterium* sp., *Corynebacterium* sp., *Pseudomonas* sp., *Rhodococcus* sp., and *Streptomyces* sp.; fungal groups include *Aspergillus* sp., *Penicillium* sp., *Cryptococcus* sp., *Rhodotorula* sp., *Fusarium* sp., were the most common isolated from stained paper manuscripts and pergamene, and they are biopigments producers on synthetic media. The seriousness of these microbial stains may be ascribed to their resistance to chemical and biological disintegration, and diffusion in fabric of colonized parchment and flax manuscripts in particular if these biopigments are extracellular in nature (Florian and Manning, 2000), thus reducing value of colonized materials (Abdel-Haliem et al., 2013).

In addition to aesthical damage, isolated microorganisms are involved in structural damage whereas our finding pointed out that *Penicillium*, *Aspergillus* & *Bacillus subtilis* are collagenase and cellulase enzymes producers, and gave a clear zone onto plates with collagen and CMC-Na as substrate. Furthermore, this enzymatic activity was detected by releasing free mono sugars of glucose and dextrins free amino acids, in particular glutamic and aspartic acid, and ammonia as end product (Sakr et al., 2013b) due to depolymerization of complex of cellulose and collagen based cultural heritage objects respectively, this effect may be assigned to enzymatic activity of collagenase and cellulase enzymes produced by a wide range of microorganism (Florian, 2006). These amino acids and free mono sugars represent carbon source for the growth and colonization of other associated microorganisms in microbiota (Konkol et al., 2013; Karbowska-Berent et al., 2011; Sterflinger et al., 2010).

In addition to fungal role in biodeterioration of colonized objects, bacteria have similar role, it has been referenced that *Bacillus pumilus* and *Bacillus firmus* are commonly isolated from paper, paperboard and recycled paper pulp due to their high enzyme activity (Logan & De Vos, 2009, 48).

Data derived from enzymatic assay confirmed that *Bacillus subtilis*, *Staphyloccus aureus Aspergillus niger* and *Penicilluim* sp. have higher collagenease enzyme activity decomposing collagen based materials such as parchment and book bindings, and this in agreement with Cappitelli & Sorlini, (2006) reported that fungi eg. *Aspergillus flavus*, *A. niger*, *Fusarium* sp., *Cladosporium*, *Scopulariopsis*, *Fusarium*, *Sporendonema*, *Ophiostoma*, *Aspergillus*, *Mucor*, *Penicillium*, *Alternaria*, *Trichoderma*, *Botryotricchum*, bacteria eg. *Bacillus subtilis*, *Staphyloccus aureus*, *Micrococcus*) are potent biodeteriogens of paper and parchment through enzymatic activity.

TiO_2 nano particles were tested against identified bacteria, and results pointed out that Bacillus (*B. firmus*, *B. pumilus*, and *B. subtilis*) are more sensitive to used nanoparticles, and varied in their sensitivity to nanoparticles, this variety could be detected even between similar strains (Ganesh Prabu et al., 2013).

On the other hand, current results revealed that microbiota showed significant differences in their resistance to tested nano particles. This may be attributed to the matter of fact that efficacy of nanoparticles on identified microorganisms should depend on size and shape of the nanoparticles (Ganesh Prabu et al., 2013), and nano metal oxides have greater surface area than their bulk counterparts, so it is expected that they might behave in a different way on interaction with microorganisms colonizing cultural heritage objects (Holtz et al. 2012).

Finally, after five generations of culturing our results documented no recovery in the treated microorganism, and it has been referenced that nanoparticles, out of them TiO_2 have fungicidal and fungi static effects against a wide range of microorganisms of bacteria, fungi and yeast (Banach et al. 2014).

5. CONCLUSION

In conclusion, this study shows that microbial colonization of fungi and bacteria caused disfiguration of colonized flax manuscripts, parchment and book bindings from specific Coptic manuscripts with green, pink and yellow pigments and showed higher enzymatic activity. TiO_2 nano particles were effective against isolated bacteria.

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