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EVALUATION OF MICROBIAL DETERIORATION OF SILVER GELATIN PHOTOGRAPHS STORED IN AN OLD PHOTOGRAPHIC ARCHIVE

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Abstract

In this work Bacillus amyloliquefaciens, which was isolated from a deteriorated photograph of an old Egyptian archive, was used to investigate the microbial role as an important factor in photograph deterioration. B. amyloliquefaciens was investigated for its proteolytic and cellulolytic activities to support its ability to degrade gelatin and cellulose, the key components of photographic material. Induced contamination of freshly prepared photographs by B. amyloliquefaciens was performed. The physical and chemical changes that happened to the photographs were evaluated using visual observation, SEM with EDX analysis and FTIR. The evaluation was performed at RH value of around 40% and at two different incubation temperatures, room temperature (24ºC) and 37ºC to determine the effect of the temperature change on the rate of deterioration. The findings showed that at the higher temperature, the rate of degradation was increased. Visual examination shows the deterioration of the gelatin layer in the contaminated sections of the photographs that were incubated at room temperature, with almost complete degradation of the entire layer for photographs incubated at 37ºC. SEM analysis revealed the presence of cracking of all contaminated images in the gelatin binder. Furthermore, the EDX study revealed a decline or total loss of silver in the cracked regions. In addition, the FTIR analysis of all contaminated photographs confirmed the damage in the gelatin layer.

Keywords*: Biodegradation; Silver gelatine; Photographic archive; SEM-EDX; FTIR analysis*

Introduction

Photographs held in public or private collections are an important object in our society since they constitute a sort of historical evidence from past times and they are considered one of the most fragile types of objects kept by museums, libraries, and archives. Silver gelatin photographs constitute three main layer structure, the first layer being the base of paper which serves as the substratum on which subsequent layers are attached. The second layer is the intermediate layer of baryta that was primarily made from gelatin and barium sulphate. The third layer is the gelatin binder, containing the photographic image's silver grains [1] and sometimes there is a fourth protective gelatin layer or overcoat [2].

There are three major forms of degradation in photographic collections: physical (structural and mechanical) damage, chemical damage, and biological damage. In biological

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damage, microorganisms represent one of the important deteriorating factors facilitated by various environmental conditions, including bad aeration, high humidity and nonhomogeneous temperature distribution in the environment surrounding the stored materials [3]. Moreover, the deteriorating action may be due to enzymatic degradation, acid corrosion and mechanical attack [4]. Alghamdi *et al.* [5] reported that the microorganisms are always found accompanying to dust particles. This was shown by Kaarakainen *et al.* [6] who reported that fungal infections in museums, collections, as well as in libraries are mostly airborne, and dust layers represent a source of fungal spores. Furthermore, Sterflinger and Piñar [4] reported that the damage caused by fungi in the paper and oil paintings on canvas was associated with cellulolytic action that breaks down cellulose fibers. In addition, various fungi were found to have proteolytic activity, such as *Aspergillus*, *Chaetomium*, *Verticillium*, *Acremonium* and *Trichoderma*. Moreover, Kraková *et al.* [7] reported that bacterial genera, including *Bacillus*, *Pseudomonas*, *Virgibacillus*, *Micromonospora* and *Staphylococcus* obtained from deteriorating parchments were found to have a pivotal role in the degradation of old books and documents made of parchment as a result of their proteolytic activities.

Regarding the biological damage, the organic constituents of the photographic materials provide a good carbon source for microbial growth [8]. Gelatin: a polymer that consists of a mixture of polypeptides obtained from animal collagen; is also an excellent rich medium for biological production. Also, while the photographic paper base mainly consists of cellulose, which is more resistant to microbial attack than gelatin substrate, it is susceptible to organisms such as fungi, bacteria and insects [9]. Besides, the gelatin binders provide a rich nutrient source for mold growth [10].

Despite the presence of silver ions in the gelatin binder which is considered an anti-microbial element, microorganisms may be able to attack the binder. This may be due to the concentration of the silver ion is not high enough to prevent microbial growth. Moreover, this is maybe due to the deteriorating effect of high temperature and high humidity on the gelatin binder [11]. On the other hand, the attractiveness of microorganisms to the gelatin layer with the presence of silver ions, maybe due to various resistance mechanisms present in the microorganisms as mentioned by various studies [12, 13]. These may include the reduction of Ag+ to the less toxic neutral oxidation state or active efflux of Ag+ from the cell by P-type adenosine triphosphatases or chemiosmotic Ag+/H+ antiporters [14]. Also, Davies [15] reported that resistance can be acquired through mutations in normal cellular genes, plasmids, or transposons.

Thus, in our research, to study the possible risk of microbial attack to photographic materials as a vulnerable type of object held by museums, libraries and archives, and to show the necessity to maintain them under certain control conditions, we evaluated the physical and chemical changes that occurred in freshly prepared photographic materials after performing induced microbial contamination using Bacillus amyloliquefaciens bacteria which was isolated from a deteriorated photograph obtained from an old private collection. The evaluation was performed at RH value around 40-50% at two different incubation temperatures, including room temperature at 24 and 37°C, to assess the impact of this variability on the deterioration rate.

Materials and Methods

Photograph collection

A private photographic collection from an old Egyptian archive in Luxor belonging to Dr. Francis Amin, a lecturer at the Faculty of Tourism and Hostels, South Valley University, Egypt, was used in this study. The collection was collected from about 50-60 years in Luxor and was stored in metallic storage until now. The photographs were in a very bad condition due to the storage, condition especially the inappropriate humidity and temperature. The most

damage was in the gelatin binder layer in the form of losses, fading, flaking, and cracking (Figs. 1 and 2).

Fig. 1. Black and white photograph samples of the used private collection showing various signs of deterioration including, gelatin binder staining and losses

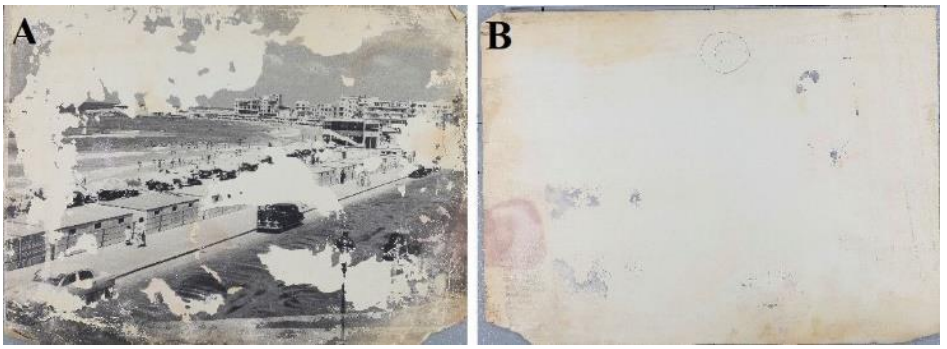

Fig. 2. Photograph C; The photograph from which *B. amyloliquefacien* was isolated, showing signs of deterioration: a) losses in gelatin binder, b) color stains in the back

Microorganism

Microorganisms used in this study were isolated at the Mycology lab, Botany and Microbiology department, Faculty of Science, Helwan University. Isolation was taken from parts of photographs with signs of biodeterioration (colored or discolored areas, or other observable textural changes) with sterile cotton swabs. The agar media chosen for this study was potato dextrose agar (PDA) a general-purpose media for molds containing $g \cdot L^{-1}$: potato, 200; dextrose, 20; agar, 15 [16], and nutrient agar media specific for bacteria containing $g \cdot L^{-1}$: peptone 5, NaCl 5, beef extract 3; agar, 15 [17]. Isolation was performed by wiping swabs on the prepared culture medium, then the plates were incubated at 25ºC for 7 days for fungi while those for bacteria were incubated at 37ºC for 72h and the RH value was around 40%.

Morphologically distinct colonies were isolated and purified onto plates with the same culture media. Further, a primary screening for the deteriorating rate of the isolated strains on freshly prepared photographs was performed and we continued the rest of the study with the potent isolate which showed the most rate of degradation of the gelatin binder (*B. amyloliquefaciens*).

Identification

DNA Isolation

Total genomic DNA was extracted using Qiagen Extraction Kit, Germany, as recommended by the manufacturer. The isolated DNA was resolved on 1% agarose gel prepared in 1X TBE (Tris-borate-ethylenediaminetetra acetic acid) buffer prepared consisting of (0.04 M Tris-borate, 0.001M EDTA (pH = 8.0) containing 0.5µg·mL^{-1} ethidium bromide (Sigma-Aldrich, USA).

PCR reaction and sequencing

The polymerase chain reaction was conducted using the MyTaqTM Red DNA Polymerase master mix (BIOLINE cat # BIO-21108, UK) as instructed by the manufacturer. Briefly, the reaction contained 1X PCR red master mix buffer, 2.0µL of 10pm·µL^{-1} of each primer, 1.0µL of DNA template (~30ng), 0.25µL of MyTaq^{TM} DNA polymerase (5U·L^{-1}), then the total volume was adjusted to 50µL using sterile water. In the thermal cycler (Biometra, Germany) the amplification reactions were performed as follows: 1st cycle of 3min at 95°C for initial denaturation, followed by 35 cycles of 20sec at 95°C (denaturation), 20sec at 55°C (annealing), 30min at 72°C (extension), and then a final extension was performed at 72°C for 10min, the reaction was kept at 4°C. PCR products were separated on 1% agarose gel. According to the manufacturer's instructions, the PCR products were purified using the Qiagen purification kit, Germany.

Sequencing data analysis

At BMR Genomics, Italy, the PCR products were sequenced using Sanger sequencer 3730xl by using bacteria primers 27F and 1492R [18] and for fungi, ITS1 and ITS4 primers were used. The obtained nucleotide sequence was aligned to the total nucleotide collection of NCBI using the Basic Local Alignment Search Tool for the nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Detection of gelatinase and cellulase activities of B. amyloliquefaciens

Gelatin and cellulose are the key components of the photographs, so gelatinase and cellulase activity has been analyzed as deterioration factors for the components of the photographs. Gelatinase has been determined using gelatin agar mediums containing (g·L^{-1}) gelatin, 20; agar, 20 and 0.2M phosphate buffer and pH adjusted to 7 [19]. Plates incubated at room temperature (24ºC) for 72h were then flooded with 10mL of acidic mercuric chloride solution [20]. The clear zone formation around the bacterial growth indicates gelatinase activity. Cellulase was determined using cellulose-agar medium containing g·L^{-1}, 0.03% urea, 0.02% KH_2PO_4, 0.14% $(NH_4)_2SO_4$, 0.03% MgSO4·7H2O, 0.5% cellulose, 0.1% peptone, 0.00001% $FeSO_4$·7H2O, 0.00016% $MnSO_4$·7H_2O, 0.00017% $ZnSO_4$·7H_2O, 0.0002% $COCl_2$, 2% agar, pH = 5 [21]. The plates with different culture media were incubated at room temperature for 72h, and further incubated at 50°C for 8h [22], then were flooded with rose-bengal (0.005%). Cellulase activity was observed by the appearance of a clear zone around the bacterial growth.

Preparation of the experimented photographs

New photographic samples were prepared by printing images on black and white photographic paper from silver gelatin glass negatives (Variant 112, FOMA BOHEMIA spol. s.r.o. (Ltd.) Company), Hradec Králové, Czech Republic). FOMA BOHEMIA is a black-and-white, variable contrast enlarging photographic paper on a baryta double weight paper base. The paper is manufactured using silver chlorobromide emulsion. The exposure time was 2.5 sec. The developer (FOMATOL LQN), as well as the fixer (Hypo), were prepared at room

temperature as recommended by the manufacturer. At room temperature, the stop bath involved rinsing in water.

Inoculum preparation

The culture of *B. amyloliquefaciens* was grown on nutrient agar slants [17] and incubated for 24h at room temperature. The culture being prepared was used to infect the photograph as seen in the next step.

Induced microbial infection on silver gelatin photographs

This experiment was performed to contaminate the freshly prepared photographic samples with *B. amyloliquefaciens* to demonstrate its effect on the degradation of the gelatin image. Under aseptic conditions, 10μL of bacterial suspension (2.0×10^5CFU/mL) was inoculated on the specimens in two areas on the gelatin side [23]. Photographic samples were incubated for 10 days at two separate incubation temperatures to determine the impact of incubation temperature variability on the rate of deterioration with keeping RH around 40%. The first group was incubated at room temperature (24^{o}C) and the second group at 37^{o}C.

Methods used to detect changes in the photographic samples following induced microbial infection

Visual observations

Monitoring of visual changes by the naked eye, including stain appearance or loss in the photo's gelatin binder. For 10 days the photo samples were observed daily to detect possible changes.

Scanning electron microscope analysis (SEM-EDX)

Scanning electron microscopy was used in combination with X-ray fluorescence (SEM-EDX). The SEM achieves a much higher resolution image for the photo samples. In addition, X-rays fluorescence (EDX) that is connected to SEM results in surface characterization with semi-quantitative element spectrum in the samples, chemical characterization of inorganic components and changes that may occur as a result of biodegradation [24]. The Zeiss LEO Supra 55VP field emission model 51-ADD001 (Oxford, USA) was used with the EDX Oxford Instrument X-act PentaFET precision model. All samples that were analyzed were first coated in gold using sputter coater EMITECH K450X. The sputtering was conducted at a working distance of 50mm at 0.05m bar and under an argon gas flow with a current of 40mA for 30sec. The analysis was carried out at SEM Laboratory, Chemical Sciences Department, University of Catania.

Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy (ATR-FTIR)

The study of the decay of photographic gelatin and paper after infection using FTIR was performed by tracking changes in functional groups and secondary gelatin structure. The instrument used was the Bruker model VERTEX 70 Spectrometer, with a resolution of 4cm^{-1}, in transmission mode in the range of 4000-400cm^{-1}. The analyzes were carried out at the Infrared Spectroscopy Laboratory, Ministry of Antiquities Research Center in Cairo, Egypt.

Results and discussion

Although the black and white picture emulsion contains silver salts and other potentially toxic compounds, the concentrations are apparently not high enough to prevent microbial growth [25].

In this study, from a primary screening of the strains isolated from the deteriorated photograph collection for their deteriorating rate on newly prepared photographs, the potent isolate which showed the highest rate of gelatin degradation in the form of gelatin loss was chosen to continue the rest of the study. The molecular recognition of the isolates was performed at BMR Genomics, Italy and the received nucleotide sequences have been aligned with the total nucleotide collection of the NCBI using the Basic Local Alignment search tool for

the nucleotide blast (https:/blast.ncbi.nlm.nih.gov/Blast.cgi) (Table 1). The potent isolate was identified as *Bacillus amyloliquefaciens* with a similarity of more than 95.7% to *Bacillus amyloliquefaciens* strain HMI57 (MH81955.1).

Table 1. The primary screening using visual observation for the deteriorating effect of the tested isolates on the gelatin binder of newly prepared photographs

Isolate name	Rate of deterioration on the gelatin binder	The sign of photograph deterioration
Bacillus methylotrophicus	++	Loss of gelatin binder
Bacillus safensis	++	Loss of gelatin binder
Bacillus amyloliquefaciens	+++++	Loss of gelatin binder
Aspergillus niger	++	Loss of gelatin binder with the appearance of spores

Gelatinase and cellulase activities of B. amyloliquefaciens

The results confirm the biodegradability of *B. amyloliquefaciens* to the photographic layers, including gelatin degradation which appeared by clear zone formation around the bacterial growth (Fig. 3a), and cellulose degradation was also observed by clear zone formation around the growth (Fig. 3b). *B. amyloliquefaciens* is known for its ability to degrade gelatin as reported in various studies [26, 27] as well as cellulose degradation as reported by [28].

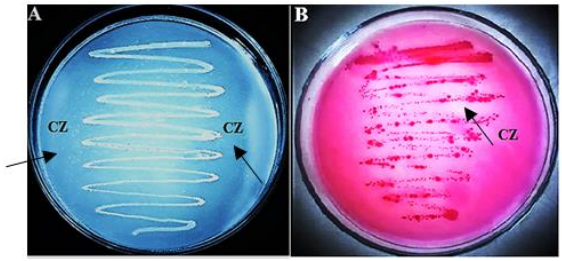

Fig. 3. Enzyme activities by *B. amyloliquefacien*. a) Gelatinase activity was shown by clear zone formation around bacterial growth, b**)** Cellulase activity was shown by disappearance of the dark pink color around the bacterial growth indicating clear zone formation. The arrows refer to the clear zone (CZ) formation around bacterial growth

Methods used to detect changes in the photographic samples following induced microbial infection

Visual observations

Visual observation by the naked eye was used to monitor the obvious changes in the photograph layers after the induced infection. With an RH value around 40%, the results of the infected photographs incubated at 37ºC showed the accelerated deteriorating rate of the examined photographs by destroying almost the entire gelatin layer within 3-5 days indicating the rapid spread of the bacterial growth on the image surface. The damage appeared after the second day of the incubation period and gradually increased until the gelatin layer almost became disappeared on the fifth day of the incubation period. This may be due to that the optimum degree of *B. amyloliquefaciens* growth known to be at 37ºC [29, 30]. Also, the photographs that incubated at room temperature at 24ºC showed a slower rate of gelatin layer degradation in the form of loss and slight disappearance in the gelatin layer at the infected areas but after a prolonged time extended to 10 days of the incubation period (Fig. 4).

Scanning electron microscope analysis

Scanning electron microscopic analysis (SEM-EDX) was performed to assess the effect of *B. amyloliquefaciens* on the surface appearance and chemical components of the photographic samples. The first analysis was carried out on the control samples to examine the

surface and the chemical components in order to observe any changes that may occur in the other samples following infection with *B. amyloliquefaciens.* SEM examination of control samples shows no damage to the surface silver grains (Fig. 5). In addition, the X-ray fluorescence showed the presence of silver, carbon and silicon, which are elements related to the paper manufacturing process. In addition, the appearance of gold may result from the coating of the photograph sample with gold prior to analysis (Fig. 6).

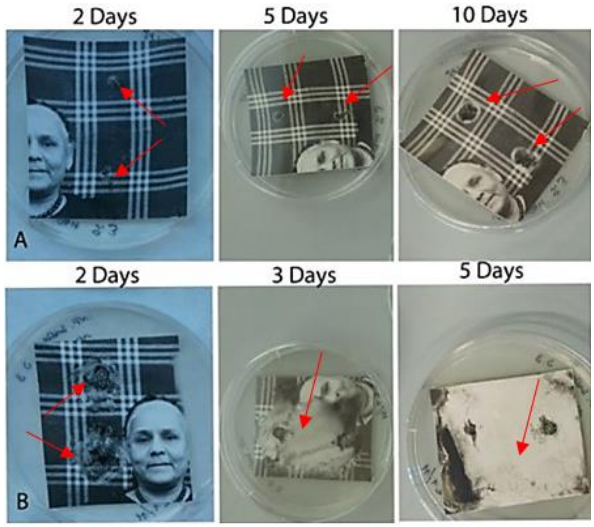

Fig. 4. The status of photo samples infected with *B. amyloliquefaciens* showing the different rate of deterioration:
A - damage of the gelatin binder appeared in the form of gelatin loss at the infected areas after 10 days of incubation period at room temperature (24ºC),
B - damage of the gelatin binder in the form of gelatin loss began after only 2 days at 37ºC showing the accelerated rate of damage with an almost complete loss after only 5 days of the incubation period, and the arrows referring to the loss areas which was greater and more spreading than those at room temperature

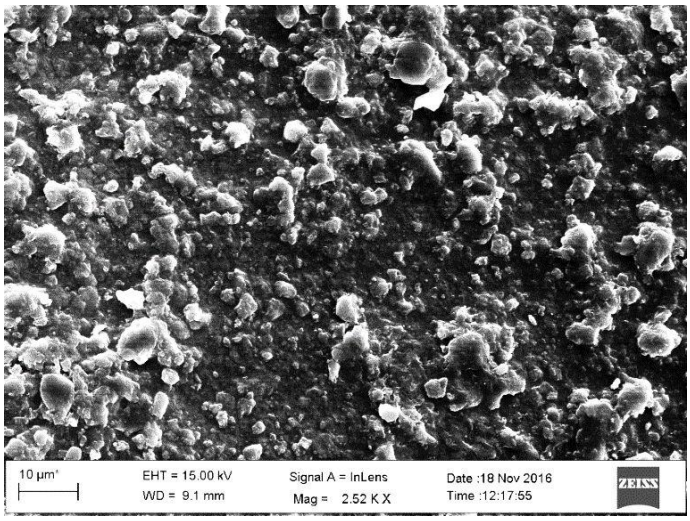

Fig. 5. SEM microscope of control photograph surface showing silver grains without any damage

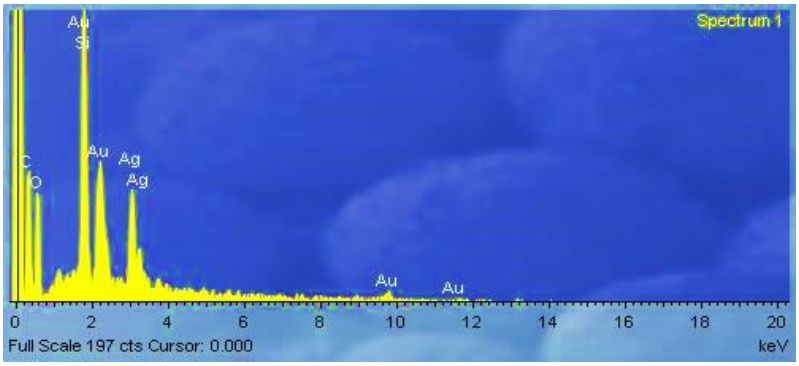

Fig. 6. XRF spectrum of control photograph showing the presence of silver as the final image material (top) and carbon, silicon which are elements related to paper making (bottom)

SEM examination of all tested *B. amyloliquefaciens* infected images revealed signs of physical damage in the form of cracks. In addition, the rod-shaped bacteria of *B. amyloliquefaciens* were clearly shown in the cracked area (Figs. 7, 8 and 9). Analysis of EDX for photographic samples contaminated with *B. amyloliquefaciens* incubated at room temperature (24ºC) (Fig. 10) revealed the presence of silver, but with a decreased amount relative to control specimens, this could be due to the use of silver by the bacteria [31]. Moreover, the detection of other elements such as calcium, carbon and silicon was related to the manufacture of the photographic image and the appearance of both barium and sulfur referring to the baryta layer [32].

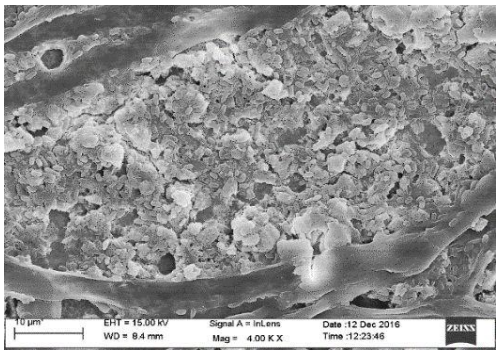

Fig. 7. SEM analysis for a photo sample infected with *B. amyloliquefaciens* incubated at room temperature(24ºC) showing cracking

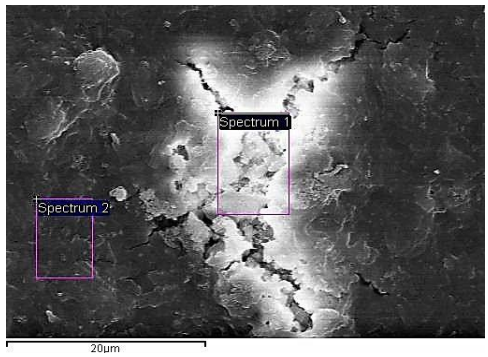

Fig. 8. SEM analysis for a photo sample infected with *B. amyloliquefaciens* incubated at 37ºC suffering from cracking

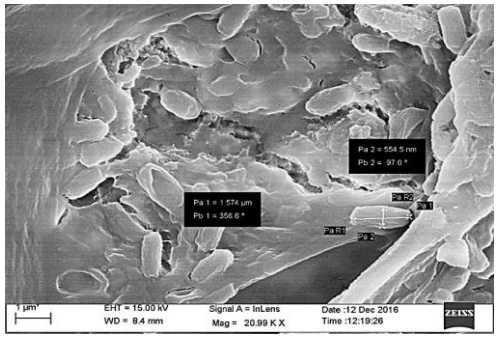

Fig. 9. Magnified part of SEM analysis for a photo sample infected with *B. amyloliquefaciens* incubated at 37ºC showing the rod-shaped bacteria within the crack

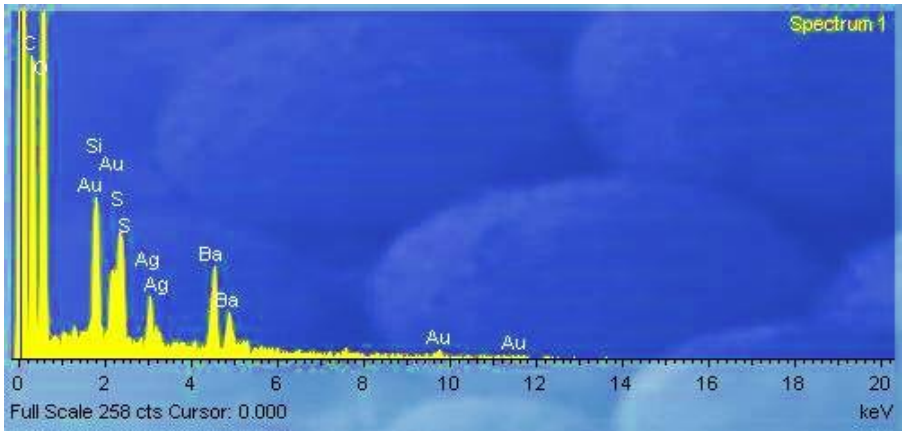

Fig. 10. XRF spectrum for photo sample infected with *B. amyloliquefaciens* incubated at room temperature (24ºC) showing the presence of silver, barium, sulfur, carbon, silicon

In addition, the EDX analysis for photographic samples contaminated with *B. amyloliquefaciens* incubated at 37ºC was performed for both spectrum areas shown in (Fig. 8) to demonstrate the cracking effect on the gelatin layer. Results in (Figs. 11 and 12) showed that there was a complete lack of silver element in the crack area represented in (spectrum 1) this could be due to the crack extending deeply into the paper base at spectrum 1 area or the use of silver by the bacteria at this point in the analysis. In addition, the presence of calcium and silicon in both measured spectra is related to their regular use in the paper manufacturing process, while barium and sulfur are related to the photograph's baryta layer [31, 32].

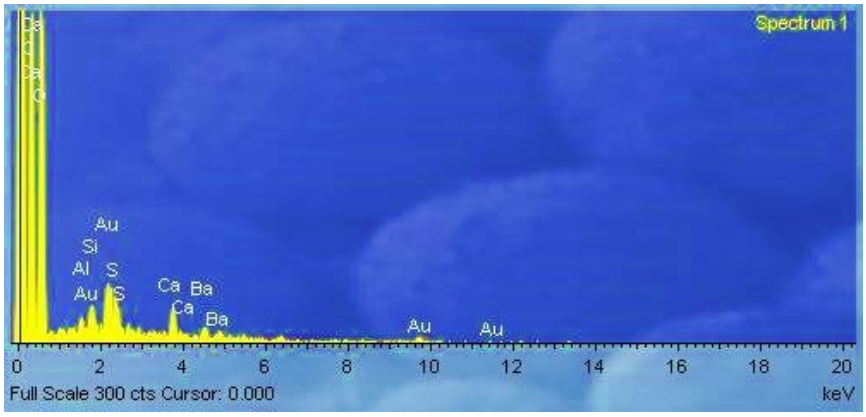

Fig. 11. XRF spectrum (1) in Fig. 8, for a photo sample infected with *B. amyloliquefaciens* incubated at 37ºC from the crack area showing the absence of silver element while the presence of carbon, calcium, silicon which were used in the paper making process, while barium and sulfur from the baryta layer

Attenuated Total Reflection - Fourier Transform Infrared Spectroscopy

According to previous studies, the results and the interpretation for the samples of the ATR-FTIR analysis were explained. Kong and Yu [33] identified nine characteristic bands of absorption of IR, amide A, B, and I−VII, in protein. The amide bands I and II are the most commonly used IR bands to illustrate the protein and peptide conformational changes [34]. The Amide I band is occurring in the 1660-1600cm^{-1} range and is associated with the C=O stretching vibration, while the Amide II band occurs in the 1565-1500cm^{-1} range and is related

to the C-H stretching and N-H bending vibration [35]. As mentioned in previous studies, there are two major degradation paths for gelatin including, hydrolysis and oxidation. Hydrolysis of gelatin appears as an increase in the OH stretching or bending frequencies found around the infrared spectrum of 3400 and 1650cm^{-1} wavenumber regions respectively. While the oxidation of gelatin leads to the formation of carbonyl compounds which would absorb in the wavenumber range of 1700-1750cm^{-1} [36].

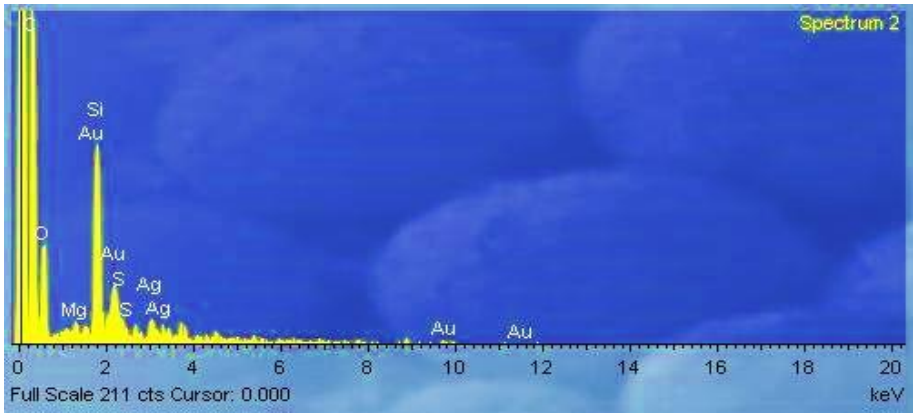

Fig. 12. XRF spectrum (2) in Fig 8, for a photo sample infected with *B. amyloliquefaciens* incubated at 37ºC showing the presence of silver from image layer, while carbon, sulfur, magnesium, and silicon used in the paper making

In our study, the results of the FT-IR spectra of all infected photographs by *B. amyloliquefaciens* showed noticeable changes in intensity of various wavenumber regions. In the case of the photographs incubated at 37ºC (Fig. 13) the changes are observed by increasing the intensities at 1637cm^{-1} of the Amide I band and at 1542cm^{-1} of Amide II band referring to gelatin oxidation. In addition, an absorption band at the wavenumber of 1731cm^{-1} appeared indicating carbonyl compound formation which refers to gelatin oxidation.

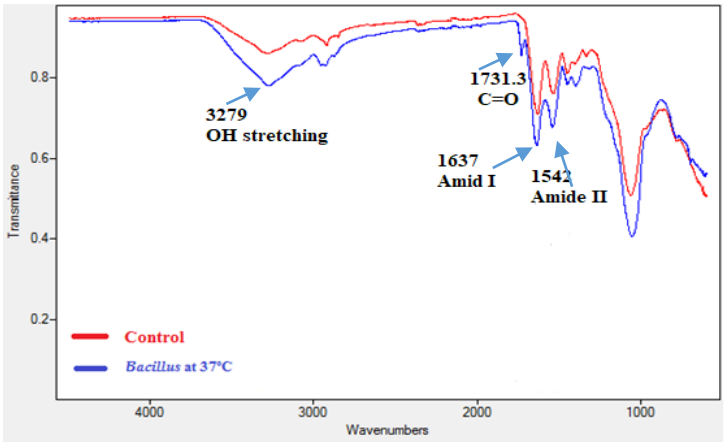

Fig. 13. ATR-FTIR spectrum for photo sample infected by *Bacillus amyloliquefaciens* sample at 37ºC. changes are observed by increasing the intensities at 1637cm^{-1} of Amide I band and at 1542cm^{-1} at of Amide II band referring to gelatin oxidation. An absorption band at the wavenumber of 1731cm^{-1} was appeared indicating carbonyl compound formation which refer to gelatin oxidation. The intensity increased at the wave number region of 3297cm^{-1} indicating increase in the OH stretching which refer to the hydrolysis of the gelatin

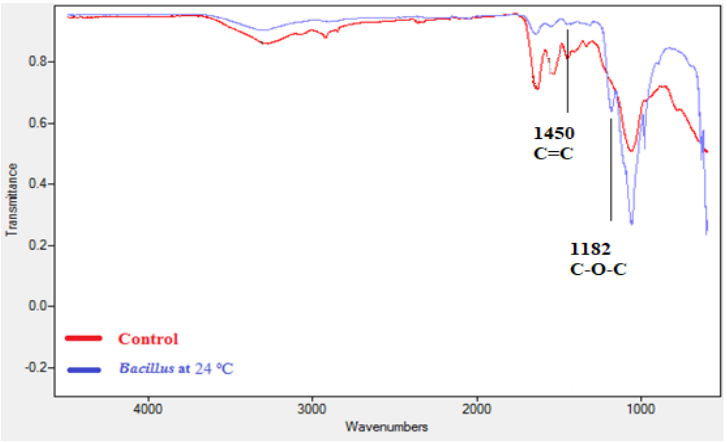

Fig. 14. ATR-FTIR spectrum for photo sample infected by *Bacillus amyloliquefaciens* at room temperature (24ºC). An absorption band at the wavenumber of 1182cm^{-1} appeared which refer to C-O-C stretching, also a decrease in the intensity at 1450cm^{-1} wave number refer to C=C bond formation indicating gelatin degradation

Furthermore, the intensity increased at the wave number region of 3297cm^{-1} indicating increase in the OH stretching which refers to the hydrolysis of the gelatin. Ali *et al.* (2012) reported that the increase in amide I band intensity is related to an increase in random coil at the expense of the ordered secondary structure. Ali *et al.* [36], and Ali and Fawzy [37], reported that the increase of the intensity of the amide I and amide II bands compared to that of the control ones suggested that the photographic emulsion has been badly affected. In the case of the photographs incubated at room temperature at 24ºC (Fig. 14), an absorption band at the wavenumber of 1182cm^{-1} appeared which refers to C-O-C stretching, also a decrease in the intensity at 1450cm^{-1} wave number refers to C=C bond formation indicating gelatin degradation. According to Oberle-Kilic *et al.* [38], Abdel-Aziz et al. [39], Yosri *et al.* [40] and Abdallah *et al.* [41] findings, it is relevant to conclude that the spectral differences result from microbiological contamination. In addition, Vivar et al. [42] reported that the biodegradation of gelatin involves the proteolytic hydrolysis of its peptide bonds.

Conclusions

This research is focused on evaluating the effect of the microbial attack on photographic materials as a valuable form of cultural heritage. The investigation and analysis methods used in this study found that the increase in the incubation temperature of the infected photograph by the bacteria *B. amyloliquefaciens* accelerated the worsening impact on the silver gelatin photographs, that was shown by visual observation of the infected photographs incubated at 37°C which showed almost complete loss of gelatin binder after 3-5 days of the incubation period. Moreover, the SEM- EDX analysis showed the deterioration in all infected photographs in the form of cracking. Moreover, the FTIR results showed that the most damage was in the gelatin layer in all the infected photographs.

So, the results explained how the storage temperature is an important factor in minimizing the microbial attack of the stored photographs, and that the lower the temperature at which the photos were stored, the lower the risk of microbial attack, recommending that the storage temperature shouldn't exceed 20°C.

Moreover, further studies must be included on the effect of RH and other environmental factors on the rate of photographs deterioration [43, 44]. Other common guidelines should be followed for photographs conservation, including placing photographs in folders inside boxes and stacks and the permanent use of air-conditioning 24h a day. the prevention of microbial

deterioration of the cultural heritage materials may be occurred by using wooden boxes or cardboard for storage that help to absorb and hold moisture with good air circulation, wearing clean cotton gloves to avoid contamination during handling and through building and insulation systems. If possible, a dehumidifier or air conditioner can be used to maintain temperature and RH at the appropriate storage condition.

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References

[1] C. Long, *Mending of fiber based silver-gelatin* ***photographs*****, Topics in Photographic Preservation: Photographic Materials Group (PMG) of the American Institute for Conservation of Historic and Artistic Works** (AIC), **3**, 1989, pp. 62–65.

[2] A.R. Duma, **School of Culture and Creative Art**, Glasgow University, 2015.

[3] A. Ritchkoff, H. Viitanen, K. Koskela, *The response of building materials to the mould exposure at different humidity and temperature conditions*, **Proceedings of Healthy Buildings, 3**, 2000, pp. 317–322.

[4] K. Sterflinger, G. Pinar, *Microbial deterioration of cultural heritage and works of art-tilting at windmills*, **Applied Microbiology and Biotechnology**, **97**, 2013, pp. 9637–9646.

[5] M.A. Alghamdi, M. Shamy, M.A. Redal, M. Khder, A. Abdel Hameed, S. Elserougy, *Microorganisms associated particulate matter*: A preliminary study, **Science of the Total Environment**, **479–480,** 2014, pp. 109–116.

[6] P. Kaarakainen, H. Rintala, A. Vepsalainen, A. Hyvarinene, A. Nevalainen, T. Meklin, *Microbial content of house dust samples determined with qPCR*, **Science of the Total Environment, 407**, 2009, pp. 4673–4680.

[7] L. Kraková, K. Chovanová, S.A. Selim, A. Šimonovičová, A. Puškarová, A. Maková, D.A. Pangallo, *A multiphasic approach for investigation of the microbial diversity and its biodegradative abilities in historical paper and parchment documents*, **International Biodeterioration & Biodegradation, 70**, 2012, pp. 117–125.

[8] C. Abrusci, A. Martin-Gonzaleza, A. DelAmo, F. Catalina, J. Collado, G. Platas, *Isolation and identification of bacteria and fungi from cinematographic films*, **International Biodeterioration & Biodegradation, 56**, 2005, pp. 58–68.

[9] S. Shrivastava, *Biodeterioration of art objects on paper and their conservation*, **Research Journal of Recent Sciences, 4**, 2015, pp. 42–46.

[10] S. Clark, **Photographic Material**, Preservation Advisory Centre, The British Library, 2009.

[11] A Casoli, S Fornaciari, *An analytical study on an early twentieth-century Italian photographs collection by means of microscopic and spectroscopic techniques*, **Microchemical Journal, 116**, 2014, pp. 24–30.

[12] C. Losasso, S. Belluco, V. Cibin, P. Zavagnin, I. Micetic, F. Gallocchio, M. Zanella, L. Bregoli, G. Biancotto, A. Ricci, *Antibacterial activity of silver nanoparticles: sensitivity of different Salmonella serovars*, **Frontiers in Microbiology, 5**, 2014, Article Number: 227. doi: 10.3389/fmicb.2014.00227.

[13] J.L. Graves, M. Tajkarimi, Q. Cunningham, A. Campbell, H. Nonga, S.H. Harrison, J.E. Barrick, *Rapid evolution of silver nanoparticle resistance in Escherichia coli*, **Frontiers in Genetics, 6**, 2015, Article Number: 42. https://doi.org/10.3389/fgene.2015.00042.
[14] D.H. Nies, *Efflux-mediated heavy metal resistance in prokaryotes*, **FEMS Microbiology Reviews, 27**, 2003, pp. 313–339.
[15] J.E. Davies, *Origins, acquisition and dissemination of antibiotic resistance determinants*, **Ciba Foundation symposium, 207**, 1997, pp. 15–27.
[16] N. da Silva, M. Taniwaki, V. Junqueira, N. Silveira, M. do Nascimento, R. Gomes, **Microbiological examination Methods of Food and Water: A Laboratory Manual**, Taylor and Francis Group, London, United Kingdom, 2013.
[17] S.P. Lapage, J.E. Shelton, T.G. Mitchell, *Media for the maintenance and preservation of bacteria (Chapter I)*, **Methods in Microbiology,** Academic Press, 1970, 3, pp. 1-133.
[18] A.F. Jereny, I.R. Claudia, S. Shobha, S.W. Jon, A.W. Brenda, J.O. Gary, *Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes*, **Applied and Environmental Microbiology, 74**, 2008, pp. 2461–2470.
[19] M.S. Ammar, S.S. Louboudy, U.M. Abdul-Raouf, *Distribution, total viable bacteria and identification of the most potent proteolytic bacterial strains isolated from Aswan City*, **Al-Azhar journal of microbiology, 11**, 1991, pp. 224–238.
[20] S.T. Cowan, **Cowan and Steel's Manual for the Identification of Medical Bacteria** (second edition), Cambridge University Press, England, 1974, pp. 193–227.
[21] S. Bland, E.E. Douglas, *Semi quantitative plate assay for determination of cellulase production by Trichoderma viride*, **Applied and Environmental Microbiology, 33**, 1977, pp. 178–183.
[22] M.E Osman, H.K Om Kalthoum, A.A El-Shaphy, *Production of cellulase and pectinase from some aquatic hyphomycetes*, **Research Journal of Microbiology, 3**, 2008, pp. 213–224.
[23] M.J. Lourenço, J.P. Sampaio, *Microbial deterioration of gelatin emulsion photographs: Differences of susceptibility between black and white and colour materials*, **International Biodeterioration & Biodegradation, 63**, 2009, pp. 496–502.
[24] M. Bučková, A. Puškárová, M.C. Sclocchi, M. Bicchieri, P. Colaizzi, F. Pinzari, D. Pangallo, *Co-occurrence of bacteria and fungi and spatial partitioning during photographic materials biodeterioration*, **Polymer Degradation and Stability, 108**, 2014, pp. 1–11.
[25] E Cserwinska, R Kovalik, *Microbiodeterioration of audiovisual collections, part 2: Microbial problems in photographic print collections. Restaurator*, **International Journal for the Preservation of Library and Archival Material, 3**, 1979, pp. 70–80.
[26] Y. Dong, H. Li, S. Rong, H. Xu, Y. Guan, L. Zhao, W. Chen, W. He, X. Gao, R. Chen, L. Li, Z. Xu, *Isolation and evaluation of Bacillus amyloliquefaciens Rdx5 as a potential biocontrol agent against Magnaporthe oryzae*, **Biotechnology & Biotechnological Equipment, 33**, 2019, pp. 408–418.
[27] Q. Jamal, Y.S. Lee, H.D. Jeon, K.Y. Kim, *Effect of plant growth-promoting bacteria Bacillus amyloliquefaciens Y1 on soil properties, pepper seedling growth, rhizosphere bacterial flora and soil enzymes*, **Plant Protection Science, 54**, 2018, pp. 129–137.
[28] S. Aslam, A. Hussain, J.I. Qazi, *Production of cellulase by Bacillus amyloliquefaciens-ASK11 under high chromium stress*, **Waste Biomass Valorization, 10**, 2017, pp. 53–61.
[29] A. Gotor-Vila, N. Teixidó, M. Sisquella, R. Torres, J. Usall, *Biological characterization of the biocontrol agent Bacillus amyloliquefaciens CPA-8: The effect of temperature, pH and water activity on growth, susceptibility to antibiotics and detection of enterotoxic genes*, **Current Microbiology, 74**, 2017, pp. 1089–1099.
[30] M. Ye, L. Sun, R. Yang, Z. Wang, K. Qi, *The optimization of fermentation conditions for producing cellulose of Bacillus amyloliquefaciens and its application to goose feed*, **Royal**

Society Open Science, 4, 2017, Article Number: 171012. http://dx.doi.org/10.1098/rsos.171012.

[31] M. Ali, M. Ali, S. Darwish, U. Saker, E. Ciliberto, E. Greco, E. Viscuzo, *Investigation and conservation of all-shenawy palace photographic collection in Mansoura, Egypt*, **Mediterranean Archaeology and Archaeometry, 15**, 2015, pp. 165–185.

[32] A. Puškárová, M. Bučková, B. Habalová, L. Kraková, A. Maková, D. Pangallo, *Microbial communities affecting albumen photography heritage: a methodological survey*, **Scientific Reports,** 2016, https://doi.org/10.1038/srep20810.

[33] J. Kong, S. Yu, *Fourier transform infrared spectroscopic analysis of protein secondary structures*, **Acta Biochimica et Biophysica Sinica, 39**, 2007, pp. 549–559.

[34] A. Adochitei, G. Drochioiu, *Rapid Characterization of peptide secondary structure by FT-IR Spectroscopy*, **Revue Roumaine de Chimie, 56**, 2011, pp. 783–791.

[35] M. Derrick, *Evaluation of the state of degradation of dead sea scroll samples using FT-IR Spectroscopy*, **American Institute for Conservation Book Paper Group Annu, 10**, 1991, pp. 49–65.

[36] M.A. Ali, M.F. Ali, M.O. Saker, A.A.E. Abdelaleem, K.I. Elnagar, *Investigations on the chemical degradation of silver gelatin prints*, **International Journal of Conservation Science, 3**(2), 2012, pp. 93–106.

[37] M. Ali, M. Fawzy, *The Effect of the Vapors of Clove and Lavender Oils on the Chemical and Optical Properties of Silver Gelatin Printsi in Wood Frames*, **International Journal of Conservation Science, 12**(3), 2021, pp. 961–976.

[38] J. Oberle-Kilic, J. Dighton, G. Arbuckle-Keil, *Chemical characterization of starch and starch: Lignin films using microattenuated Total Reflectance Fourier Transform Infrared Spectroscopy (micro-ATR FTIR)*, **Trends in Biomaterials & Artificial Organs, 26**, 2012, pp. 107–109.

[39] O. Abdel-Aziz, M. Mahmoud, Y. Abo-Elfath, S. Abdel-Aziz, M. Samir, M. Ali, *Evaluation of Conventional Treatments for Mirrored Silver Gelatin Prints: Experimental and Applied Study*, **International Journal of Conservation Science, 10**(1), 2019, pp. 81–96.

[40] M. Yosri, M. Ali, F. Stanco, K. Talaat, *Restoration of Silver Gelatin Prints in The Digital Era: An Innovative Approach*, **International Journal of Conservation Science, 9**(3), 2018, pp. 375–388.

[41] M. Abdallah, M. Ali, A. Belal, F. Stanco, F. Mohareb, M. Ali, *Conservation of the Heinz and Georges Leichter Dry Plate Collection, Egypt: Case Study*, **International Journal of Conservation Science, 11**(2), 2020, pp. 463–484.

[42] I. Vivar, S. Borrego, G. Ellis, D.A. Moreno, A.M. García, *Fungal biodeterioration of color cinematographic films of the cultural heritage of Cuba*, **International Biodeterioration & Biodegradation, 84**, 2013, pp. 372-380.

[43] A. Yosri, N. Mohamed, A. Atef, O. Atef, R. Attia, M. Ali, *Analytical Study on the Effects of Pollutants on Silver Gelatin Prints*, **International Journal of Conservation Science, 11**(1), 2020, pp. 39–50.

[44] R. Shaheen, M. Fouad, O. Saqr, S. Reda, A. Labeeb, *Assessment of the Photo-Chemical Degradation of Silver Gelatin Photograph Print-Out*, **International Journal of Conservation Science, 11**(4), 2020, pp. 1093–1102.

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