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ORIGINAL ARTICLE

Investigation on microbial deterioration of exquisite collection of old manuscripts in Iran

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ABSTRACT

Background and Objectives: The present study was to evaluate the microbial diversity inhabiting biodeteriorated precious manuscripts of the Holy Quran placed in one of the repositories of the Library of Astan Quds Razavi (AQR), and its relation to the air microbial diversity.

Materials and Methods: Three non-invasive sampling methods, culture-based techniques, and molecular identification were used to investigate the microorganisms involved in deterioration. To investigate the air microbial quality and its role in the destruction of the repository objects, air samples were taken from six different points inside the repository. Biomodeling studies were designed to verify the impact of microbial isolates.

Results: 14 fungal isolates were obtained from three deteriorated ancient Quran manuscripts. The most frequently isolated fungi from the different substrates were *Aspergillus* spp. and *Penicillium* spp. In the air, the prevalence across fungal genera was rather uniform. 30 species of the identified bacteria were collected from three manuscripts. The results obtained in the present study showed that the bacterial species from different genera belonged to three phyla: *Proteobacteria* (n = 2), *Actinobacteria* (n = 4), and *Firmicutes* (n = 24). The paper strips were artificially colonized by *Aspergillus* sp., *Penicillium chrysogenum*, and *Talaromyces diversus* producing spots which were visible to the naked eye. In the scanning electron microscopy images, the colonization of the selected organism was observed.

Conclusion: The characteristics of paper inoculated artificially with these microbial isolates confirmed their deteriorating effects. Based on molecular identification, the similarity of fungal and bacterial species isolated from both substrates and air samples suggest the direct relationship between microorganisms from the air and those isolated from the manuscripts.

Keywords: Fungi; Bacteria; Microbial deterioration; *Proteobacteria*; Library

INTRODUCTION

The effect of microorganisms, particularly fungi, in the biodeterioration of papers, parchments, and artifacts of cultural heritage is well known (1). Globally, damage to paper products and library materials by these microorganisms is a major concern. Despite proper storage conditions such as low storage temperature, humidity, and good ventilation, deterioration still occurs in library materials (1, 2).

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According to prior research, deterioration can be attributed to different microorganisms such as fungi and bacteria that can deposit and colonize on repository documents (3-5). Under certain environmental conditions like suitable temperature, relative humidity, light, and nutrition, the spores of fungi settle down on the surfaces of shelves or other library materials and then germinate and form colonies (6). Bacterial growth often starts after the growth of fungi, which is due to the changes in the microenvironments and ecological niches. Living organisms cause biological deterioration on different substrates of library material including any book, picture, photograph, painting, drawing, map, newspaper, magazine, pamphlet, manuscript, document, letter and record, audiovisual materials in any format, catalog cards and electronic data. There are different types of biodeterioration, which can be categorized in the form of physical/mechanical, and biological damages (4, 5).

The Central Library of Astan Quds Razavi (AQR) is one of the country's most important cultural institution and the precious treasuries of the recorded culture in Iran (7). Before 1979, the cultural activities of Astan Quds were limited to four libraries including Mashhad Central Library, Malik Library, Waziri Library and Harandi Library (7, 8). It stores and conserves precious historical manuscripts, including the manuscripts of the Holy Quran, old papers, parchments, and ancient books (7). Having over ninety thousand handwritten books, this center is one of the principal resources for researchers. Although there have been many reports related to the microbial deterioration of the historical objects and repository materials, studies on the microbial communities from the old manuscripts of the Holy Quran have not been explicitly considered (7, 8). Therefore, screening of deterioration of these kinds of manuscripts is a major area of interest in the field of cultural heritage and can benefit the managers to promote the maintenance condition of these materials.

The aim of this study was to investigate the microbial diversity of deteriorated papers and parchments of several old manuscripts of the Holy Quran. Furthermore, we examined the role of airborne microorganisms in contamination of library objects and to evaluate the repository air microbial qualities. Understanding the microbial communities in the repositories could provide valuable information for the best practice in maintenance management.

MATERIALS AND METHODS

Site and sample characteristics. Sampling was performed in one of the repositories of AQR, Iran. The repository covers an area of about 200 m^2, is located on the underground floor. It contains 3841 copies of the Quranic manuscript, 11383 Quranic pamphlets. In the repository, the air ventilation system works continually. In addition, there is an air buffering room between this repository and the general library. The temperature (19ºC) and relative humidity (RH 55) inside of this repository were continuously measured.

Three deteriorated samples belonging to a valuable collection of the old manuscript of the Holy Quran were selected.

1) Sample S1 (Fig. 1a): The sample was made of parchment. The exquisite parchment manuscript of the Holy Quran was written in the ninth century (AD)/third century (AH).

2) Sample S2 (Fig. 1b): The Holy Quran belongs to the tenth century [fifth century (AH)] and sampling was performed from both leather cover and papers.

3) Sample S3 (Fig. 1c): Quranic pamphlet from paper belongs to the tenth century (327 AH).

In all samples, although the restoration including removing dust, brushing and repairing the damaged pages was carried out, microbial biodeterioration was observed.

Book sampling. Three Quranic manuscripts, which showed signs of biodeterioration were sampled by a non-destructive sampling procedure to isolate the deteriorating fungi and bacteria. For each contaminated area, two sterile swabs, which were slightly moistened in sterile normal saline were rubbed over the deteriorated spots on bindings. After that, one of the swabs was directly cultured onto agar plates and the other was put in sterile normal saline for 2 hours و and then inoculated onto agar plates. The third sample was taken by sterile nitrocellulose membranes (Sigma-GE10600002, AmershamTM Protran® Western blotting membranes, nitrocellulose; 0.45 m pore size, 2*2 mm diameter), which were gently pressed on the visible spots on the contaminated areas for 10 s, and then transferred into the media (1). For all samples, the following three different media were prepared and used to isolate, purify and identify microorganisms. According to Christensen et al., (1959), Malt Extract Agar (MEA, Scharlau) with 15% NaCl to be a medium with low water activity, and 0.05 g/l chlor-

Fig. 1. S1, S2, S3: The manuscripts of the old Holy Quran dated back to ninth, tenth century, which belongs to the Library of AQR.

amphenicol (Sigma) to limit the bacterial growth, Sabouraud Dextrose Agar (SDA, Scharlau) with 0.05 g/l chloramphenicol, and Trypticase Soy Agar (TSA, Scharlau) with 0.2% nystatin (Iran) to limit fungal development, were used (1, 7).

Air sampling. To determine the bacterial and fungal contamination of air repository, the passive sedimentation sampling method was carried out. Inside the repository, three replicas open plates were placed at six different points for 30 minutes, including on the way of two air ventilation inlets, two air ventilation outlets, and two places in the center of the room at 70 cm and 150 cm from the floor on the bookshelves and near the deteriorated objects. To compare the amount of fungal and bacterial contamination of main repository air with air buffering room, only one place, 140 cm above the floor, was sampled.

Cultivation and identification experiments. As mentioned before, in each type of sampling method, three different media including SDA, MEA and TSA were used. These media were incubated at 28°C for 7-14 days and checked daily. To obtain the pure culture, colonies with different morphology were transferred onto the new plates of TSA and SDA for bacteria and fungi, respectively (2).

All of the purified fungal colonies were morphologically investigated using an optical microscope (Olympus, Japan). The slide culture method was used to observe vegetative and sexual structures.

Molecular analysis. For molecular identification of fungi and bacteria, its DNA was extracted from pure cultures using fungal DNA extraction kit (Pouya Gene Azma Co., Iran) and a bacterial DNA extraction kit (Sina Clon, Iran).

For fungal identification, the ITS1, the ITS2 region and 5.8S rRNA gene were amplified using the primer pair ITS1 (5'CTTGGTCATTTAGAGGAAGTAA3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') (8). The polymerase chain reaction (PCR) was performed using an amplicon PCR master mix according to the manufacturer's recommendation (Amplicon, Korea) with 2 µl DNA and 12/5 pmol of each primer. The thermocycling program was as follows: 94°C for 5min; 35 cycles at 92°C for 1 min, 54°C for 1 min, 72°C for 1 min followed by a final extension step at 72°C for 10 min. The PCR products were sequenced and the raw results were analyzed by Lasergene ver. 6.0 software (DNASTAR, Madison, WI, USA) and then compared with

the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The 16S rRNA gene was applied to amplify bacterial DNA using the universal primer pair 27f (5'AGAGTTTGATCMTGGCTCAG3') and 1492r (5' GGTTACCTTGTTACGACTT3') (9). The PCR reaction mixture was prepared following the manufacturer's instructions (Amplicon, Korea), including 25µL containing 0.75 µL of each primer and 0.5 µL DNA as template. The thermocycling program was as follows: initial denaturation at 94ºC for 2 min, followed by 25 cycles of 1 min denaturation at 94ºC, 1 min annealing at 58ºC, and 50s extension at 72ºC. Five minutes at 72ºC were used as a final extension step.

Biomodeling of paper deterioration. To evaluate the deteriorating impacts of fungi, three isolated fungi were selected and grown on cellulose paper strips. The spores of *Aspergillus* sp., *Penicillium chrysogenum* and *Talaromyces diversus* were obtained from 3-day-old cultures. For this step, the spores were suspended in 3 ml of distilled sterile water containing 0.02% tween 80 (Merck-Schuchardt, Germany), prepared according to Pinzari et al. 2006 (10). Then, 50 µL of this suspension was inoculated on two points of paper strips under a laminar flow cabinet in the sterile condition. Each inoculated paper strip was placed in a 20 cm glass plate and incubated at 25ºC under moisture conditions (10).

The fungi growing on paper strips were directly observed with a stereomicroscope (Olympus, Japan). Then, SEM images of these papers were taken by an LEO 1450 VP from the Carl-Zeiss Electron Microscopy Group (Jena, Germany). Before SEM observation, the samples were sputter-coated with gold.

For examining the deteriorating impacts of bacteria, the synthetic medium was prepared. It contained the following ingredient per 100ml: 0.05g KH_2PO_4, 0.02g $MgSO_4.7H_2O$, 0.01g NH_4NO_3, 0.002g $FeSO_4$, 0.005g $Ca(NO_3)_2$, and then 3ml of this media was distributed in the test tubes (11). The sterile paper strip was placed in each tube, and then 10 µl from the prepared bacterial broth culture was inoculated in it, and then incubated at 38ºC for 3-7 days. Two species of *Cellulosimicrobium aquatile* were selected to inoculate on this step.

Before SEM observation, the paper samples were washed with dH_2O 2 till 5 times to rinse the minerals. After that the paper strips were dried and fixed using 0.28% glutaraldehyde in 0.1M buffer phosphate (pH 7.2) for 2 hours at 4ºC. Finally, papers were dehydrated with serial dilution of alcohol and then were sputtered with gold.

RESULTS

Identification of the deteriorating microorganisms. The deteriorated ancient manuscripts of the Holy Quran from AQR are shown in Fig. 1. As shown in the figure, the parchment sample (S1) showed different deterioration signs compare to the other two samples with purple stains. These various stains may be due to the different chemical properties of the substrates and their related microorganisms. The PCR results for fungi detection was shown in Fig. 3. As presented in Table 1, fourteen fungal isolates were obtained from three deteriorated ancient Quran manuscripts. As results, 35.7% of the isolates were from S1, 42.9% and 21.4% of isolates were from the S2 and S3 samples, respectively. Based on the microscopic and molecular phylogenetic identification, the genera of the isolated fungi were as follows: *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., and *Cladosporium* spp. Based on the ITS region analysis, *Talaromyces diversus* and *Arachinotus littoralis* were also identified from S1 and S2, respectively. Fig. 2 demonstrates *Alternaria*

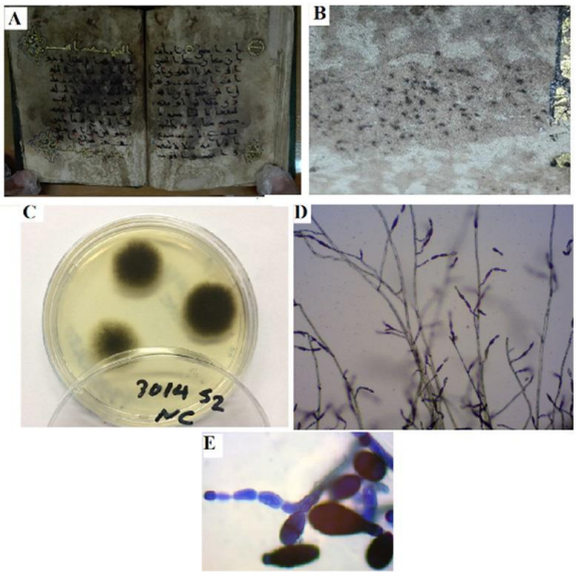

Fig. 2. A: Contaminated area of sample S3. B: Contaminated area of Sample S3 with high magnification, C: The pure culture of *Alternaria* spp. on SDA, D: Light microscopic images of *Alternaria* spp. with 40x magnification, and E: Light microscopic images of *Alternaria* spp. with 100×.

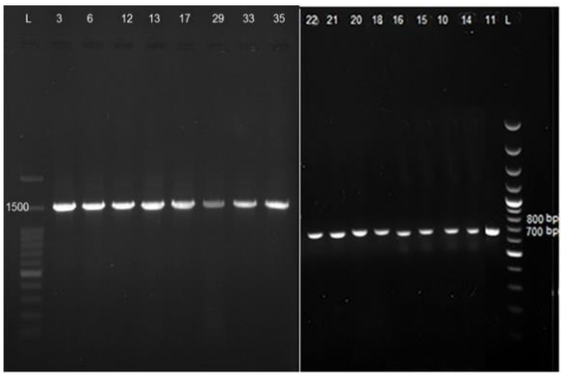

Fig. 3. The PCR result of ITS-rDNA region of fungal samples (left) and 16S rRNA of bacteria (right). Line L in both images indicate to ladder.

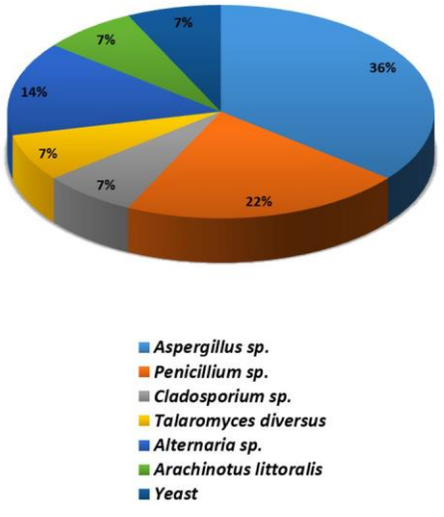

Fig. 4. Pie chart of distribution of fungi isolated from manuscripts.

sp. isolated from S3. The pie chart in Fig. 4 shows that *Aspergillus* sp. was frequently isolated, followed by *Penicillium* spp. and *Alternaria* spp.

Table 1 provides an overview of fungal isolates from different substrates. *P. chrysogenum*, *Cladosporium cladosporioides*, *Talaromyces diversus*, and *Aspergillus* spp. was detected from S1 sample.

All of the fungi grown on MEA with 15% NaCl, belonged to the parchment sample (Table 2). In the present study, two fungal isolates from parchment were identified as *P. chrysogenum.*

As indicated in the Table 2, 30 species of the identified bacteria were collected from three manuscripts. 53.3% species were isolated from S1, and 16.7% and 30% species were isolated from S2 and S3, respectively. The results obtained in the present study showed that the bacterial species from different genera belonged to three phyla: *Proteobacteria* (n = 2), *Actinobacteria* (n = 4), and *Firmicutes* (n = 24).

The distribution of the other bacterial isolates is illustrated in Fig. 5. As predicted, most of the bacteria were isolated on TSA. However, five isolates including three *B. atrophies* strains, one *Staphylococcus warneri* strain, and a *S. epidermidis* strain were isolated on SDA with chloramphenicol supplement, which these strains may be chloramphenicol-resistant.

Investigation of air samples. In AQR repository, the temperature and relative humidity of the air is controlled at 19 ± 1°C and 55%, respectively. The results of the fungal and bacterial cultures isolated from six points in the repository and one point in the buffering room were presented in Tables 3 and 4, respectively.

As presented in Table 3, 17 fungal isolates were detected on three different nutrient media including MEA, SDA, and TSA. Most fungi including *Purpureocillium lilacinum*, *Talaromyces diversus*, *Cladosporium* sp. and *Aspergillus* sp. were isolated on MEA (41.2%). Other fungal genera including *Penicillium*, *Cladosporium*, *Alternaria*, and *Aspergillus* were also isolated from the air of the buffering room (Table 3).

Sequence analysis revealed that the most similarity ranging from 97 to 100% in NCBI database, belonged to the phylum Firmicutes. Furthermore, spore-forming *Bacillus* sp. was present in all samples (Table 4). Five species were detected in repository air identified as Gram-positive cocci and they were related to the genus *Staphylococcus. Bacillus zhangzhouensis* was identified in the air of both the repository and the buffering room.

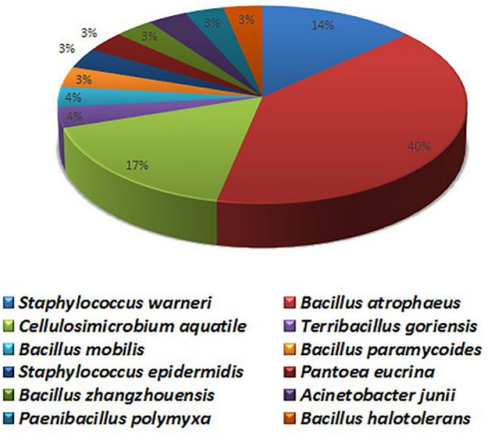

Fig. 5. Pie Chart of distribution of bacterial isolated from manuscripts.

Table 1. Fungal diversity from various sampling methods on holy handwritten Qurans.

	MEA	SDA	TSA	MEA	SDA	TSA	Frequency
	S1			S2			
S1	*Penicillium chrysogenum** *Cladosporium cladosporioides*	*Aspergillus* spp.		*Talaromyces diversus*		-	5 (35.7%)
S3		Yeast	*Alternaria* spp.	*Alternaria* spp.		-	3 (21.4%)
S2		*Penicillium corylophilum*	*Arachinotus littoralis*		*Aspergillus proliferans*** *Aspergillus flavus*	-	6 (42.9%)
Total	3 (21.4%)	3 (21.4%)	2 (14.3%)	2 (14.3%)	4 (28.6%)	-	14 (100%)

S1: Sample 1 (parchment). S2: Sample 2 (Manuscript belong to the tenth century). S3: Sample 3 (Manuscript belongs to the tenth century). *Two isolates of *Penicillium chrysogenum* were detected. **Three isolates of *Aspergillus proliferans* were detected.

Table 2. Bacterial diversity from various sampling methods on holy handwritten Qurans.

	SDA	TSA	SDA	TSA	Frequency
	S1		S2		
S1	*Staphylococcus warneri*	*Staphylococcus warneri* *Bacillus halotolerans,* *Bacillus atrophaeus*** *Paenibacillus polymyxa* *Cellulosimicrobium aquatile* *Bacillus zhangzhouensis* *Acinetobacter junii*	*Bacillus atrophaeus*	*Bacillus atrophaeus* *Cellulosimicrobium aquatile**	16 (53.3%)
S3	*Bacillus atrophaeus***	*Terribacillus goriensis* *Bacillus atrophaeus* *Bacillus mobilis*	*Bacillus atrophaeus*	*Bacillus paramycoides* *Bacillus atrophaeus*	9 (30%)
S2	*Staphylococcus epidermidis*		*Staphylococcus warneri*	*Staphylococcus warneri* *Pantoea eucrina*	5 (16.7%)
Total	5 (16.67%)	12 (40%)	4 (13.3%)	9 (30%)	30 (100%)

S1: Sample 1 (parchment). S2: Sample 2 (Manuscript belong to the tenth century). S3: Sample 3 (Manuscript belongs to the tenth century). *Four isolates of *Cellulosimicrobium aquatile* were detected. **Three isolates of *Bacillus atrophaeus* were detected.

Similar bacteria (i.e., *Staphylococcus warneri, Bacillus halotolerans, Bacillus atrophaeus, Bacillus zhangzhouensis* and *Terribacillus goriensis*), and three fungi (i.e., *Penicillium* spp., *Talaromyces diversus* and *Cladosporium* spp.) were isolated from both substrate and air samples (Tables 1-4). The most frequency bacterial species that can be isolated by culture method in both sample, belonged to the *Bacillus* genus.

The accession numbers of the bacterial and fungal isolates are available in NCBI, and including MK093005, MK093006, MK093029, MK093022, MK093021, MK093023, MK093024, MK093007, MK093026, MK093010, MK093009, MK093011, MK093008, MK093033, MK093032, MK093013, MK093012, MK093017, MK093015, MK093016, MK093027, MK093019, MK093028, MK093030, MK093020, MK093018, MK093014, OR041589, OR041518, OR041505, OR041502, OR039904, OR039815,OR039835, OR039815, OR039835, OR039904, OR041502, OR041505, OR041518, OR041589, OR083072, OR083077, OR083077, OR083141 and OR083143.

Biomodeling of paper strips. The paper strips were artificially colonized by *Aspergillus* sp., *Peni-*

Table 3. Distribution of fungi isolated from air.

	MEA	SDA	TSA	Frequency
AIn E	*Purpureocillium lilacinum*			2 (11/76%)
	Talaromyces diversus			
AEx E	*Talaromyces diversus*		*Cladosporium* sp.	2 (11/76%)
AIn D		*Pseudozyma* sp.		1 (5/88%)
AEx D				0
AS H70			*Penicillium* sp.	1 (5/88%)
AS H150				0
AL H140	*Cladosporium* sp. *	*Penicillium chrysogenum**	*Cladosporium* sp.	11 (64/7%)
	Aspergillus sp.	*Alternaria* sp.	*Alternaria* sp.*	
	Cladosporium herbarum	*Cladosporium* sp.		
Total	7 (41/18%)	5 (29/41%)	5 (29/41%)	17 (100%)

AIn E: On the way of air ventilation inlets end of the repository. AEx E: On the way of air ventilation outlets end of the repository. AIn D: On the way of air ventilation inlets near the repository door. AEx D: On the way of air ventilation outlets near the repository door. AS H70 and AS H150: Center of room at 70 cm and 150 cm from the floor. AL H140: buffering room at 140 cm above the floor. *Two isolates were detected.

Table 4. Distribution of bacteria isolated from air samples.

	SDA	TSA	Frequency
AIn E		*Staphylococcus warneri**	
		Bacillus atrophaeus	5 (19.23%)
		*Terribacillus goriensis**	
AEx E	*Bacillus atrophaeus*	*Staphylococcus warneri*	
		Bacillus zhangzhouensis	4 (15.4%)
		Psychrobacillus sp.	
AIn D	*Bacillus halotolerans*	*Bacillus zhangzhouensis*	
		Staphylococcus hominis subsp.	3 (11.54%)
		Novobiosepticus	
AEx D	*Bacillus halotolerans*	*Paenibacillus polymyxa*	
	Bacillus atrophaeus	*Psychrobacillus* sp.	4 (15.4%)
AS H70			0
AS H150	*Staphylococcus warneri*	*Bacillus halotolerans*	
	Bacillus zhangzhouensis		4 (15.4%)
	Psychrobacillus sp.		
AL H140		*Bacillus halotolerans*	
		Bacillus atrophaeus	
		Bacillus zhangzhouensis	
		Bacillus amyloliquefaciens	6 (23%)
		Psychrobacillus sp.	
		Acinetobacter junii	
Total	7 (27%)	19 (73%)	26 (100%)

AIn E: On the way of air ventilation inlets end of the repository. AEx E: On the way of air ventilation outlets end of the repository. AIn D: On the way of air ventilation inlets near the repository door. AEx D: On the way of air ventilation outlets near the repository door. AS H70 and AS H150: Center of room at 70 cm and 150 cm from the floor. AL H140: buffering room at 140 cm above the floor. *Two isolates were detected.

cllium chrysogenum and *Talaromyces diversus* produced spots that are visible with the naked eye. One strip was selected for scanning electron microscope (SEM) observation. Fig. 6 shows *Cellulosimicrobium aquatile* cultured on synthetic media with paper strips as the only carbon source. SEM images with different magnifications were presented in Fig. 7 for fungi and bacteria.

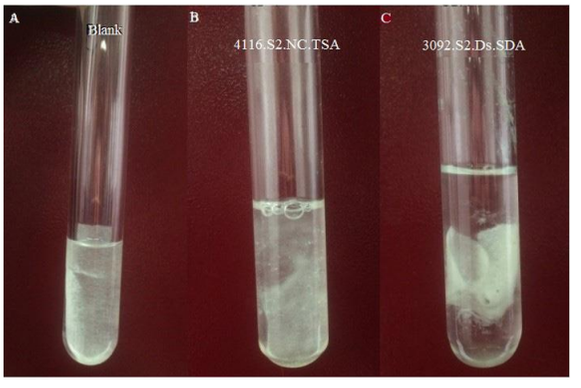

Fig. 6. The prepared synthetic medium containing sterile paper strip, A. Blank, B, C. Bacteria.

DISCUSSION

Identification of the deteriorating microorganisms. The library of AQR is one of the great ancient libraries with precious written heritage in the world. There is an extensive and valuable collection of old manuscripts of the holy Quran and documents stored in the AQR. Therefore, the preservation of these precious collections is very important.

In present study, the most fungal frequency was seen on the S1 manuscript. In prior studies, *Aspergillus*, *Cladosporium*, *Penicillium*, and *Alternaria* had been isolated from different substrates such as parchment, wood-pulp paper, laid paper, and old manuscripts (12, 13). Likewise, *Aspergillus* sp. and *Penicillium* sp. followed by *Alternaria* sp. were most dominant isolates on paper and parchment manuscript (13-16). Bergadi et al. (2013) investigated the ancient manuscripts in an old Medina library in the city of Fez, and identified different fungal isolates including *Aspergillus* and *Penicillium chrysogenum*, which were able to produce cellulases (Bergadi et al.,

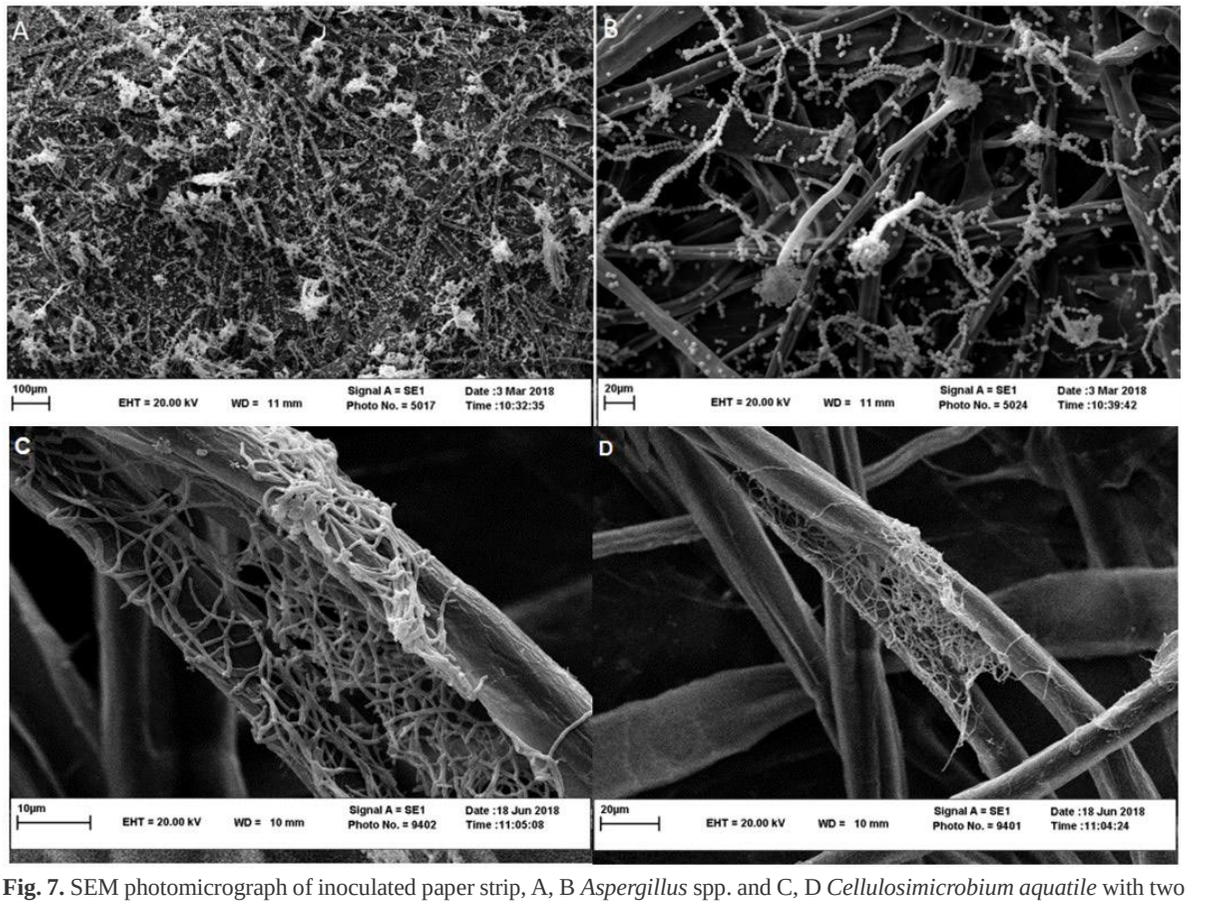

Fig. 7. SEM photomicrograph of inoculated paper strip, A, B *Aspergillus* spp. and C, D *Cellulosimicrobium aquatile* with two different magnifications.

2013). So far, more than 200 species of cellulolytic fungi have been reported from libraries, and most of them belong to the genera of *Aspergillus, Penicillium, Cladosporium,* and *Fusarium* (Nardi 2019).

Among the fungi isolated from three Quran manuscripts, *Arachinotus littoralis* has not been previously isolated and reported from biodeteriorated paper.

Salts such as sodium chloride (NaCl) were reported to inhibit microbial activity on parchments. Salts can be added in the first step of the manufacturing process of paper (17). Interestingly, all fungi grown on MEA with 15% NaCl, belonged to the parchment sample. Thus, these are halotolerant or halophilic fungi (14). Furthermore, parchment is a material made of animal skins, and mainly consists of collagen. *P. chrysogenum* could play a crucial role in the deterioration of this type of parchment substrate due to produce alkaline proteases with strong proteolytic activity (17, 18).

Among the *Firmicutes phylum*, the most frequently bacterial genus was *Bacillus* (n = 18) and *B. atrophies* was detected with high frequency (40%). *Cellulosimicrobium aquatile* can grow under salt concentrations of up to 13% (19), thus its isolation from salty substrates such as parchments was not unexpected. Recently, this non-spore-forming bacterium, which has glucosidase activity was isolated from Panagal reservoir in India (19). The present study is the first report on *Cellulosimicrobium aquatile* from deteriorated parchment. We found that *B. halotolerans* was detected only in parchment substrate. Therefore, the chemical properties of the parchment could explain the presence of this halotolerant bacterium.

As previously mentioned, the comparison between the parchment sample (S1) and non-parchment samples (S2 and S3) showed typical purple or damage stains (Fig. 1). According to a report by Pinar et al. (2015), these kinds of spots can be attributed to collagen lysis via microorganisms using the collagen as a carbon source (14). In addition, these discolorations can be related to halophilic and halotolerant microorganisms.

Investigation of air samples. In previous studies was reported that the air microbial contamination levels had a direct influence on biodeterioration processes in library and repository materials. As reported previously, *Purpureocillium lilacinum* has been isolated from stone, concrete and mortar substrates (20). Its isolation from the repository air sample can be due to its presence in the airborne compositions (21). *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., and *Aspergillus* spp. have been reported to be the most common in indoor environments (22, 23). The colonies of these fungi produce millions of spores, which can be released easily into air. The abundance of fungi from the buffering room air was greater than the repository air. The low number of isolates of the repository can be attributed to its efficiently controlled environment.

Among the bacteria, most of the strains isolated from air samples were Gram-positive bacilli. *Bacillus zhangzhouensis* that was identified in the air of both the repository and the buffering room, can be isolated from the habitats with a broad range of temperature (8 till 45°C) and saline environments (0 till 12% NaCl w/v) (24).

In an increasing number of cultural heritage studies, *Bacillus* species was detected in a variety of paintings, manuscripts, papers and library materials (25-28). A possible explanation for the high frequency of *Bacillus* can be attributed to their ability to form endospores, allowing them to maintain their viability under unfavorable environmental conditions over a long time. Compared to other airborne bacterial species, *Bacillus* endospores in air can settle down on the open plates and germinate rapidly. Based on the other reports, these bacteria are predominant in indoor air, and their spores can not only settle down on the different library materials, but also persist on the environment in form of endospores (28). In addition, *Bacillus* species can attack to cellulose fibers such as paper products, parchments, paints and other related substrates. These bacteria with producing cellulase use paper as a sole carbon source (5).

Biomodeling of paper strips. The results of this study showed that fungi and bacteria can biodeteriorate paper, made of cellulose. From biomodeling method is used to simulate almost the real conditions of paper degradation by microorganisms. Three different species of fungi, *Aspergillus* sp., *Penicillium* sp., and *Cellulosimicrobium* sp. were inoculated on paper and incubated. The paper was then examined for the presence of spots and signs of deterioration. The findings suggested that these microorganisms can cause significant damage to paper and break down cellulose and other components of paper. This experiment can be proper to understand the deterioration mechanisms, and to be tested the efficiency

of new methods of prevention or control of biodeterioration. The findings provides the support for this idea that there is a direct relationship between the microorganisms in air and those isolated from manuscripts. AQR library is a major library that houses valuable manuscripts, thus deserves special attention. Due to the lack of a quarantine room and suitable cleaning of the donated documents in AQR library, the source of the external contaminants should be more considered.

CONCLUSION

The present case study was designed to evaluate the deterioration of the manuscripts of the old Holy Quran housed in one of the repositories in AQR. In general, fungi and bacteria were isolated from the samples of the old Holy Quran. The repository air of AQR library can be one of the sources of biodeteriorating agents in the indoor environment. Furthermore, the isolated microorganisms showed worse deteriorating impacts in laboratory models due to more concentration of spores, and proper incubation conditions. Because many microorganisms which cannot grow on culture media may involve in the deterioration of papers, thus it is necessary in future studies to apply culture-independent methods to identify the microbial communities in the air and manuscripts.

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REFERENCES

1. Borrego S, Lavin P, Perdomo I, Gómez de Saravia S, Guiamet P. Determination of indoor air quality in archives and biodeterioration of the documentary heritage. *ISRN Microbiol* 2012; 2012: 680598.
2. Micheluz A, Manente S, Tigini V, Prigione V, Pinzari F, Ravagnan G, et al. The extreme environment of a library: Xerophilic fungi inhabiting indoor niches. *Int Biodeterior Biodegrad* 2015; 99: 1-7.
3. Kalaskar PG, Zodpe SN. Biodeterioration of library resources and possible approaches for their control. *Int J Appl Res* 2016; 2: 25-33.
4. Borrego S, Guiamet P, Gómez de Saravia S, Batistini P, Garcia M, Lavin P, et al. The quality of air at archives and the biodeterioration of photographs. *Int Biodeterior Biodegrad* 2010; 64: 139-145.
5. Lavin P, Gómez de Saravia SG, Guiamet PS. An environmental assessment of biodeterioration in document repositories. *Biofouling* 2014; 30: 561-569.
6. Sterflinger K, Pinzari F. The revenge of time: Fungal deterioration of cultural heritage with particular reference to books, paper and parchment. *Environ Microbiol* 2012; 14: 559-566.
7. Christensen CM, Papavizas GC, Benjamin CR. A New Halophilic Species of Eurotium. *Mycologia* 1959; 51: 636-640.
8. White TJ, Bruns TD, Lee SB, Taylor JW (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand DH, Sninsky JJ, White TJ. Eds. PCR Protocols: A Guide to Methods and Applications, Academic Press, New York, 315-322.
9. Gurtner C, Heyrman J, Piñar G, Lubitz W, Swings J, Rölleke S. Comparative analyses of the bacterial diversity on two different biodeteriorated wall paintings by DGGE and 16S rDNA sequence analysis. *Int Biodeterior Biodegrad* 2000; 46: 229-239.
10. Pinzari F, Pasquariello G, de Mico A. Biodeterioration of paper: A SEM study of fungal spoilage reproduced under controlled conditions. *Macromol Symp* 2006; 238: 57-66.
11. Nico G, Setiawan A, Elisabeth F, Ratna E, Lindayani, Setiawan GNA (2019). Effectiveness of UV Rays on The Ability of Selected Soil Bacteria in Decomposing LDPE Plastic.
12. Dunca SI, Tanase C, Padurariu C, Balaes T, Ardelean E, Puica NM. Study of the contaminating microbiota of old paper supports. *Eur Sci J* 2014; 3: 237-251.
13. Mesquita N, Portugal A, Videira S, Rodríguez-Echeverría S, Bandeira AML, Santos MJA, et al. Fungal diversity in ancient documents. A case study on the Archive of the University of Coimbra. *Int Biodeterior Biodegrad* 2009; 63: 626-629.
14. Piñar G, Sterflinger K, Pinzari F. Unmasking the measles-like parchment discoloration: Molecular and microanalytical approach. *Environ Microbiol* 2015; 17: 427-443.
15. Gutarowska B. Moulds in biodeterioration of technical materials. *Folia Biol Oecol* 2014; 10: 27-39.
16. Romero SM, Giudicessi SL, Vitale RG. Is the fungus Aspergillus a threat to cultural heritage? *J Cult Herit* 2021; 51: 107-124.
17. Reed R. (1975). The Nature and Making of Parchment. Leeds, England: The Elmete Press.
18. Omrane Benmrad M, Moujehed E, Ben Elhoul M, Mechri S, Bejar S, Zouari R, et al. Production, purifi-

cation, and biochemical characterization of serine alkaline protease from Penicillium chrysogenium strain X5 used as excellent bio-additive for textile processing. *Int J Biol Macromol* 2018; 119: 1002-1016.
19. Reddy Sultanpuram V, Mothe T. Draft genome sequence of cellulosimicrobium aquatile 3bp, a glucosidase active bacterium and the type strain of a novel species. *Biosc Biotechnol Res Asia* 2017; 14: 783-784.
20. Deng JX, Paul NC, Sang HK, Lee JH, Hwang YS, Yu SH. First report on isolation of Penicillium adametzioides and Purpureocillium lilacinum from decayed fruit of Cheongsoo grapes in Korea. *Mycobiology* 2012; 40: 66-70.
21. Luangsa-Ard J, Houbraken J, van Doorn T, Hong S-B, Borman AM, Hywel-Jones NL, et al. *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. *FEMS Microbiol Lett* 2011; 321: 141-149.
22. Visagie CM, Yilmaz N, Renaud JB, Sumarah MW, Hubka V, Frisvad JC, et al. A survey of xerophilic *Aspergillus* from indoor environment, including descriptions of two new section *Aspergillus* species producing eurotium-like sexual states. *MycoKeys* 2017; 19:1-30.
23. Osman ME, Awad AH, Ibrahim YH, Ahmed YF, Abo-Elnasr A, Saeed Y. Air microbial contamination and factors affecting its occurrence in certain book libraries in Egypt. *Egypt J Bot* 2017; 57: 93-118.
24. Liu Y, Lai Q, Du J, Shao Z. *Bacillus zhangzhouensis* sp. nov. and *Bacillus australimaris* sp. nov. *Int J Syst Evol Microbiol* 2016; 66: 1193-1199.
25. López-Miras Mdel M, Martín-Sánchez I, Yebra-Rodríguez A, Romero-Noguera J, Bolívar-Galiano F, Ettenauer J, et al. Contribution of the microbial communities detected on an oil painting on canvas to its biodeterioration. *PLoS One* 2013; 8(11): e80198.
26. Phulpoto AH, Qazi MA, Mangi S, Ahmed S, Kanhar NA. Biodegradation of oil-based paint by *Bacillus* species monocultures isolated from the paint warehouses. *Int J Environ Sci Technol* 2016; 13:125-134.
27. Gorbushina AA, Heyrman J, Dornieden T, Gonzalez-Delvalle M, Krumbein WE, Laiz L, et al. Bacterial and fungal diversity and biodeterioration problems in mural painting environments of St. Martins church (Greene-Kreiensen, Germany). Int Biodeterior. *Biodegrad* 2004; 53: 13-24.
28. Karbowska-Berent J, Górny RL, Strzelczyk AB, Wlazło A. Airborne and dust borne microorganisms in selected Polish libraries and archives. *Build Environ* 2011; 46: 1872-1879.