Pol. J. Environ. Stud. Vol. 29, No. 4 (2020), 2679-2695
DOI: *10.15244/pjoes/111236* ONLINE PUBLICATION DATE: 2020-02-17

Original Research

Effect of Fungi on the Destruction of Historical Parchment and Paper Documents

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Received: 9 May 2019
Accepted: 24 July 2019

Abstract

Fungal frequency and diversity were studied in historical, parchment and paper documents using Illumina sequencing. In total, 156 494, 52 451 and 41 615 sequences were obtained from three samples. Documents were colonized by 225 taxa. Glomeromycota, Zygomycota, Ascomycota and Basidiomycota were represented by 1, 8, 131 and 83 taxa, respectively. Fungal communities included plant pathogens, epiphytes or endophytes of a wide range of plants and possibly opportunistic plant pathogens, saprotrophs common in soil, on decaying leaves, needles, wood and on other plant material, human pathogens, animal and nematode pathogens, entomopathogenic taxa, mycoparasites, white and black yeast-like taxa, taxa with antagonistic and medicinal properties, lichenized fungi, food contaminants, common contaminants of indoor, built environments, taxa on herbivore dung, keratin-degrading taxa, xerophilic taxa, and an endangered fungi included in the Red List of Threatened Species. A non-destructive and non-invasive method for quantitative characterization of parchment deterioration, based on spectral measurements, was used for evaluating the scale of damage.The collagen-to-gelatin ratio, estimated from the synchronous fluorescence spectra of the studied samples and of pure collagen and gelatin, was suggested for characterizing parchment condition. Analysis of fluorescence peaks indicated the moderate stage of deterioration of the studied documents.

Keywords: diversity, fungi, ITS1/2 rDNA, historical documents, parchment

Introduction

Microorganisms (bacteria, archaea and fungi), together with lichens and insects, due to their biodeteriorative potential, damage items of cultural heritage. Fungi play an important role in archives and libraries where stored objects are made of paper or parchment. The main component of paper is cellulose, and of parchment it is inner collagen type I, with its typical fibre arrangement and external gelatin layers. Paper and parchment can be sources of energy, carbon and nitrogen for numerous microorganisms.

Bacteria are the main contaminants of parchment due to their preferences for higher pH [1-6]. Filamentous fungi also play an important role in biodeterioration due to their preferences for lower temperature and higher humidity, often present in archives. Typical fungal deterioration is caused by the slow-growing, often mitosporic, cellulolitic or proteolytic

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and often xerophilic Ascomycetes from the genera *Alternaria, Aspergillus, Cladosporium, Chaetomium, Chrysosporium, Paecilomyces, Penicillium, Phoma* or *Stachybotrys* [5, 7-16]. On contaminated objects, fungi produce coloured stains (species of *Chaetomium*, *Epicoccum*, *Monoascus*), and emit strong odours (*Trichoderma* spp.) or toxic compounds (*Stachybotrys* spp.).

Fungal contamination usually arises from airborne inoculum. Colonization is determined by the nature and chemical composition of the material, climate, conditions of storage (i.e., poor ventilation and temperature changes, which cause water condensation), the mode and frequency of document usage and methods of cleaning.

Deterioration may cause distortion and deformation of documents. They become misshapen, the text fades, and contaminated areas may be covered with white films and multi-coloured spots and residues, called 'foxing' [17].

Biodeterioration and mechanical damage result from chemical degradation by fungal enzymes (cellulases, collagenases), chemical modification of inorganic components and production of pigments and organic acids penetrating and discoloring the document structure [10, 15, 18, 19].

The need for prevention and correct protection of historical objects is emphasized by conservators, archivists, and museologists [11, 20]. Implementation of appropriate remedial measures must be preceded by detailed research, including detection and recognition of the potential microbial hazard.

The aims of this study were: (i) to determine the abundance and diversity of fungi in historic parchment and paper documents in two archives in Poland, and (ii) to consider the destructive activity of the fungi detected in the prevailing environmental conditions.

Detection and identification of fungi was based on fungal genomic ITS rDNA studied with the Illumina technique. The ITS rDNA sequencing for studies of fungal contamination on historical documents was applied earlier [15, 21].

Materials and Methods

Description of Documents

Analyses of three samples from two documents were made in 2016.

Document 1 is a book by Ioannes de Pineda SI entitled "Ad suos in Salomonem commentarios … libri octo", printed in 1613 in Mainz, Germany. Until 1881 the book was in a cloister in the Warmian-Masurian Voivodeship in northeastern Poland. In 1882 the book was moved to an attic of the parish building and stored in a wooden cabinet, with seasonal variations in temperature and humidity. Currently the book is stored in the Old Documents Archive in Toruń Diocese. Its binding is made of cardboard covered with parchment. Its pages are made of handmade paper. The page edges are dyed with ultramarine. The book shows symptoms of deformation and fungal contamination (powdery or fluffy white, grey, reddish, brown or black deposits in numerous places on the binding and pages). The text is faded.

Document 2 is a torah originating from the 19th century from Zamość County in eastern Poland. In 1940-1970, wrapped in paper, it was stored in a wooden cabinet with exposure to seasonal variations of temperature and humidity. Since 2014 it has been stored in acid-free paper in a wood and glass cabinet in the storeroom of the Foundation for the Preservation of Jewish Heritage in Poland, in Warsaw. Its scroll is made of calf parchment treated with chalk and polished. Two wooden shafts are made of maple wood. Four crowns attached to shafts are made of lime wood. The wooden elements are polychromed and dyed with orpiment, ultramarine, English red, red lead, white, green and black natural paints and calcium carbonate. The handwriting on the scroll is done with a metal-tannic ink. Defects have been repaired with calfskin glued with gum Arabic or joined with natural or partly dyed linen threads. The torah parchment is creased and cracked vertically and horizontally. Its deterioration occurs mostly in gum Arabic-treated parts. There is no visible fungal contamination.

Quantitative Characterization of Parchment Deterioration

Both documents and the control currently made parchment sample were independently assessed by spectral measurements at 25°C [22]. The fluorescence was examined with a spectro-fluorimeter equipped with a surface-analysis accessory. Spectra were measured from both sides. The excitation wavelengths were 243 nm and 298 nm. The excitation and emission bandwidths were 4 nm. Fluorescence was correlated with deterioration of documents. Quantitative analysis was based on the comparison of spectra of collagen, gelatin and our samples. The collagen-to-gelatin ratio (C/G ratio) and the percentage of collagen were estimated.

Sampling for Mycological Analysis

Samples 1 and **2** from **Document 1** were respectively from white, fluffy deposits present on the parchment of the book binding and from greyish powdery deposits present on the book pages. **Sample 3** from **Document 2** was from cream-beige-brown spots covered with powdery deposits present on gum Arabic-treated parts of the torah scroll. Samples were collected with a sterile scalpel from the surface of 1 cm^2 areas with no text.

Molecular Identification
DNA Extraction, Amplification and Sequencing

DNA was extracted with a Bead-Beat Micro AX Gravity Kit (A & A Biotechnology). ITS 1/2 rDNA amplification was performed with fungus-specific primers gITS7 (5' GTG ART CAT CGA RTC TTT G 3`) [23] and iITS4 (5`TCC TCC GCT TAT TGATAT GC 3). The PCR reaction mixture (25 µl) consisted of 12.5 µl of 2x MixPCR, 0.2 µM of each primer, 1.5 µl of purified and diluted DNA and 10.6 µl of water. The PCR reaction was performed under the following conditions: denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 56ºC for 30 s and elongation at 72ºC for 30 s, and a final elongation at 72ºC for 7 min. The amplicons were sequenced using the Illumina technique.

Bioinformatics Analysis

A table of operational taxonomic units (OTUs) was prepared by PIPITS, version 1.2.0 [24]. Read-pairs were joined with PEAR, version 0.9.6 [25], filtered with a quality threshold of q = 30 by FASTX-toolkit, version 0.0.13 (http://hannonlab.cshl.edu/fastx_toolkit/index.html), converted to Fasta format and merged into a single file. Prepared sequences were dereplicated and subregions of ITS were selected with the use of ITSx, version 1.0.11 [26]. Unique sequences and those shorter than 100 bp were removed. Remaining sequences were clustered with 97% sequence identity. The resulting representative sequences for each cluster were subjected to chimera detection and removal using the UNITE UCHIME reference dataset, version 6.0 (https://unite.ut.ee/index.php). The input sequences were then mapped onto the representative sequences and taxonomy assigned using an RDP Classifier, version 2.10.2 against UNITE fungal ITS reference database, version 11.2 [27]. Sequences were identified by comparison with reference sequences from the National Center for Biotechnology Information (NCBI) database. The abundance of fungi was defined as the number of OTUs in a sample. Frequency of an individual taxon was defined as the percentage (%) of OTUs in the total number of OTUs. Diversity was defined as the number of species in a sample.

Statistical Analysis

Diversity in microbial communities was calculated for each community. Analyses were based on the abundance of fungi and taxonomic composition of communities [28]. Diversity is indicated by Margalef's diversity index (DMg) and Shannon's diversity index (H'). Evenness and dominance are indicated by Simpson's diversity index (D), Shannon's evenness index (E) and Berger-Parker's dominance index (d). The similarity between fungal communities is determined by Sorensen's qualitative similarity index (CN). Similarity and relationships among fungal communities are shown by a heat map and Venn diagram (Fig. 1, Table 1).

Results

Compared to the control, aging and deterioration of documents caused a decrease in fluorescence intensity, the spectral shift of the main peak and an overall change in the fluorescence spectral features. For 298 nm excitation samples 1-3 overall fluorescence peaks were at 440-460 nm, and control sample fluorescence peaked at 400 nm. For 243 nm excitation the second broad weak bands of samples 1-3 emerged at 400-500 nm, and control sample fluorescence peak was at 300 nm. The spectrum of collagen consisted of two well-defined peaks at 305 and 345 nm and an additional broad band with a maximum at 440 nm. The spectrum of gelatin has a well-defined peak at 440 nm and additional broad bands at 305 and 345 nm. Spectra of samples 1 and 3 shifted toward pure gelatin. The C/G ratio in sample 1 was 0.8-8.2 and in sample 3 was 1.2-8.0. The average percentage of collagen in samples 1 and 3 was 8.9 and 9.1.

Three samples yielded, respectively, 156 494, 52 451 and 41 615 raw sequences (Table 1). There were 156 393 = 99.93%, 52 355 = 99.81% and 40 689 = 97.77% sequences of culturable fungi; 9 = 0.006%, 37 = 0.071% and 548 = 1.317% sequences of non-culturable fungi; and 37 = 0.024%, 19 = 0.036% and 375 = 0.901% sequences of organisms with no reference sequence in NCBI.

Documents were colonized by at least 225 fungal taxa (Table 1). Glomeromycota, Zygomycota, Ascomycota and Basidiomycota were represented

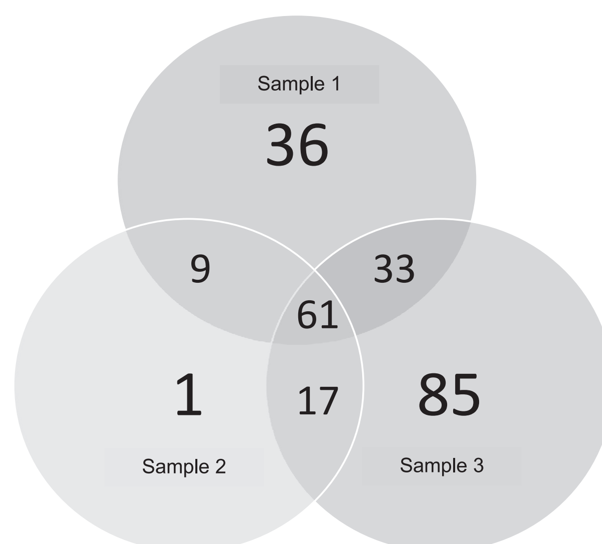


Fig. 1. Venn diagram: overlapping circles illustrate the similarities, differences, and relationships between fungal communities, with numbers of taxa shown.

Table 1. Frequency of taxons in documents.

No.	Taxon	Category	Order	Similarity to the reference sequence	Document 1				Document 2	
					Sample 1		Sample 2		Sample 3	
					Book parchment binding		Paper page		Torah parchment scroll	
					No. OTUs	Frequency (%)	No. OTUs	Frequency (%)	No. OTUs	Frequency (%)
	Glomeromycota				**1**	**0.001**	**0**	**0**	**0**	**0**
1.	*Entrophospora contigua* nom. Nud		Diversisporales	97%	1	0.001	0	0	0	0
	Zygomycota				**3234**	**2.067**	**148**	**0.282**	**1261**	**3.030**
1.	***Mortierella elongata*** Linnem. + ***M. gamsii*** Milko + *M. horticola* Linnem. + *M. macrocystis* W. Gams + ***M. parvispora*** Linnem. + *M. sclerotiella* Milko + *Mortierella* sp.	saprotroph	Mortierellales	98-100%	9	0.006	67	0.128	847	2.035
2.	*Mucor plumbeus* Bonord.	saprotroph, animal pathogen, indoor contaminant	Mucorales	99%	3225	2.061	81	0.154	414	0.995
	Ascomycota				**151875**	**97.048**	**52071**	**99.276**	**34501**	**82.905**
1.	***Acremonium charticola*** (Lindau) W. Gams + *Acremonium* sp.	saprotroph	Hypocreales	99%	27138	17.341	37	0.071	76	0.183
2.	***Alternaria alternata*** (Fr.) Keissl. + ***A. obovoidea*** (E.G. Simmons) Woudenb. & Crous + *Alternaria* sp.	epiphyte, endophyte, latent plant pathogen	Pleosporales	99%	1628	1.040	419	0.799	4620	11.102
3.	*Arachniotus aurantiacus* (Kamyschko) Arx	saprotroph	Onygenales	99%	141	0.090	0	0	68	0.163
4.	*Arthrinium phaeospermum* (Corda) M.B. Ellis + *Arthrinium* sp.	human pathogen	Incertae sedis	100%	7	0.004	62	0.118	438	1.053
5.	Ascomycota			97-99%	6252	3.995	46	0.088	59	0.142
6.	*Aspergillus albertensis* J.P. Tewari + *A. candidus* Link + *A. halophilicus* M. Chr., Papav. & C.R. Benj.+ ***A. jensenii*** Jurjević, S.W. Peterson & B.W. Horn + *A. penicillioides* Speg. + *A. proliferans* G. Sm. + ***A. tardicrescens*** Sklenar, Houbraken, Zalar & Hubka + *A. tennesseensis* Jurjević, S.W. Peterson & B.W. Horn + ***A. versicolor*** (Vuill.) Tirab. + ***Aspergillus* sp.**	saprotroph	Eurotiales	98-100%	12659	8.089	4420	8.427	475	1.141
7.	*Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud	black yeast-like, epiphyte, endophyte, latent plant pathogen	Dothideales.	100%	6	0.004	4	0.008	160	0.384

Table 1. Continued.

8.	***Beauveria bassiana*** (Bals.-Criv.) Vuill. + *B. caledonica* Bissett & Widden	entomopathogenic	Hypocreales	100%	30	0.019	180	0.343	2258	5.426
9.	*Bionectria rossmaniae* Schroers.	saprotroph	Hypocreales	99%	25	0.016	130	0.248	2211	5.313
10.	*Botryosphaeria stevensii* Shoemaker	plant pathogen	Botryosphaeriales	99%	1	0.001	14	0.027	171	0.411
11.	*Botrytis cinerea* Pers	plant pathogen	Helotiales	99%	58	0.037	462	0.881	6572	15.792
12.	*Cadophora* sp.	plant pathogen	Helotiales	99%	2	0.001	1	0.002	19	0.046
13.	*Candida boleticola* Nakase + *C. sake* (Saito & M. Ota) Uden & H.R. Buckley ex S.A. Mey. & Ahearn + *C. tropicalis* (Castell.) Berkhout	human pathogen	Saccharomycetales	99%	0	0	1	0.002	40	0.096
14.	*Capronia semi-immersa* (Cand. & Sulmont) Unter. & F.A. Naveau	black yeast-like	Chaetothyriales	99%	4	0.003	0	0	0	0
15.	*Cenangium ferruginosum* Fr.	plant pathogen	Helotiales	99%	0	0	0	0	5	0.012
16.	***Chaetomium globosum*** Kunze + ***C. iranianum*** Asgari & Zare + ***C. murorum*** Corda + *C. umbonatum* D. Brewer	saprotroph, human pathogen	Sordariales	100%	84852	54.221	39177	74.693	1619	3.890
17.	*Chalara* sp.	saprotroph	Microascales	99	13	0.008	123	0.235	1844	4.431
18.	Chaetothyriales		Chaetothyriales	97	3	0.002	0	0	12	0.029
19.	*Chrysosporium undulatum* P. Vidal, Guarro & Ulfig	keratin degrader	Onygenales	100	8	0.005	0	0	0	0
20.	*Cladosporium herbarum* (Pers.) Link + *C. iridis* (Fautrey & Roum.) G.A. de Vries + *C. pseudocladosporioides* Bensch, Crous & U. Braun + *C. ramotenellum* K. Schub., Zalar, Crous & U. Braun + *Cladosporium* sp.	epiphyte, endophyte, latent plant pathogen, indoor contaminant	Capnodiales	98-100%	36	0.023	13	0.025	541	1.300
21.	*Clonostachys rosea* (Preuss) Mussat	epiphyte, endophyte, latent plant and nematode pathogen, mycoparasite	Hypocreales	100%	2	0.001	27	0.051	455	1.093
22.	*Coniothyrium olivaceum* Bonord.	epiphyte, endophyte, latent plant pathogen	Pleosporales	98%	1	0.001	1	0.002	43	0.103
23.	*Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora	with medicinal properties	Hypocreales	100%	1	0.001	2	0.004	46	0.111
24.	*Cyclaneusma minus* (Butin) DiCosmo, Peredo & Minter	plant pathogen	Rhytismatales	100%	1	0.001	2	0.004	73	0.175
25.	*Cytospora* sp.	plant pathogen	Diaporthales	99%	0	0	0	0	17	0.041
26.	*Debaryomyces prosopidis* Phaff, Vaughan-Mart. & Starmer	food contaminant	Saccharomycetales	100%	1	0.001	0	0	218	0.524
27.	*Desmazierella acicola* Lib.	saprotroph	Pezizales	99%	1	0.001	0	0	0	0

Table 1. Continued.

28.	*Didymella macrostoma* (Mont.) Qian Chen & L. Cai + *D. pinodes* (Berk. & A. Bloxam) Petr	plant pathogen	Pleosporales	100%	1	0.001	0	0	22	0.053
29.	*Diplodia allocellula* Jami, Gryzenh, Slippers & M.J. Wingf.	epiphyte, endophyte, latent plant pathogen	Botryosphaeriales	99%	0	0	11	0.021	221	0.531
30.	Dothideomycetes			97%	0	0	0	0	2	0.005
31.	*Epicoccum nigrum* Link	epiphyte, endophyte, latent plant pathogen	Pleosporales	100%	13	0.008	37	0.071	345	0.829
32.	*Erysiphe syringae* Schwein.	plant pathogen	Erysiphales	99%	2	0.001	1	0.002	5	0.012
33.	*Exophiala xenobiotica* de Hoog, J.S. Zeng, Harrak & Deanna A. Sutton + *Exophiala* sp.	black yeast-like, human pathogen	Chaetothyriales	99%	34	0.022	0	0	36	0.087
34.	*Fusarium armeniacum* (G.A. Forbes, Windels & L.W. Burgess) L.W. Burgess & Summerell + ***F. avenaceum*** (Fr.) Sacc. + ***F. sporotrichioides*** Sherb.	plant pathogen	Hypocreales	100%	53	0.034	107	0.204	2152	5.171
35.	Herpotrichiellaceae		Chaetothyriales	98%	0	0	1	0.002	18	0.043
36.	*Heydenia alpina* Fresen.	saprotroph	Incertae sedis	100%	10	0.006	0	0	0	0
37.	*Hyaloscypha aureliella* (Nyl.) Huhtinen	saprotroph	Helotiales	100%	1	0.001	0	0	39	0.094
38.	*Hypogymnia physodes* (L.) Nyl.	lichenized fungus	Lecanorales	100%	0	0	0	0	7	0.017
39.	*Infundichalara microchona* (W. Gams) Réblová & W. Gams	saprotroph	Helotiales	100%	0	0	0	0	9	0.022
40.	*Isaria farinosa* (Holmsk.) Fr.	entomopathogenic		100%	1	0.001	2	0.004	46	0.111
41.	*Lecanicillium longisporum* (Petch) Zare & W. Gams	saprotroph, entomopathogenic	Hypocreales	100%	0	0	0	0	55	0.132
42.	*Lecythophora* sp.	saprotroph, human pathogen	Coniochaetales	100%	256	0.164	0	0	32	0.077
43.	Leotiomycetes			100%	0	0	5	0.010	106	0.255
44.	*Leptodontidium trabinellum* (P. Karst.) Baral, Platas & R. Galán + *Leptodontidium* sp.	epiphyte, endophyte, latent plant pathogen	Helotiales	99-100%	0	0	0	0	49	0.118
45.	*Leptospora rubella* (Pers.) Rabenh.	epiphyte, endophyte, latent plant pathogen	Incertae sedis	99%	0	0	0	0	15	0.036
46.	*Lophodermium pinastri* (Schrad.) Chevall.	plant pathogen	Rhytismatales	100%	5	0.003	3	0.006	79	0.190
47.	*Microascus brevicaulis* S.P. Abbott + *Microascus melanosporus* (Udagawa) Woudenb. & Samson	epiphyte, endophyte, latent plant pathogen, indoor contaminant	Microascales	99%	329	0.210	55	0.105	3	0.007
48.	*Microsphaeropsis proteae* (Crous & Denman) Crous & Denman	plant pathogen	Pleosporales	100%	0	0	0	0	5	0.012

Table 1. Continued.

49.	*Mollisia* sp.	epiphyte, endophyte, latent plant pathogen	Helotiales	99%	1	0.001	0	0	0	0
50.	*Mycosphaerella tassiana* (De Not.) Johanson	plant pathogen	Mycosphaerellales	99%	26	0.017	0	0	67	0.161
51.	*Naevala perexigua* (Roberge ex Desm.) K. Holm & L. Holm	epiphyte, endophyte, latent plant pathogen	Helotiales	99%	0	0	0	0	9	0.022
52.	*Nectria cinnabarina* (Tode) Fr.	plant pathogen	Hypocreales	99%	0	0	1	0.002	18	0.043
53.	*Neoascochyta exitialis* (Morini) Qian Chen & L. Cai	plant pathogen	Pleosporales	99%	13	0.008	0	0	0	0
54.	*Neocatenulostroma germanicum* (Crous & U. Braun) Quaedvl. & Crous	plant pathogen	Capnodiales	99%	0	0	0	0	14	0.034
55.	*Neophaeomoniella eucalypti* Rooney-Lath. & Crous	epiphyte, endophyte, latent plant pathogen	Phaeomoniellales	100%	0	0	0	0	1	0.002
56.	*Neophaeothecoidea proteae* (Crous) Quaedvl. & Crous	plant pathogen	Capnodiales	99%	0	0	0	0	3	0.007
57.	*Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams	plant pathogen	Helotiales	99%	0	0	0	0	19	0.046
58.	*Ophiognomonia sogonovii* D.M. Walker	epiphyte, endophyte, latent plant pathogen	Diaporthales	100%	2	0.001	0	0	0	0
59.	*Ophiostoma novo-ulmi* Brasier	plant pathogen	Ophiostomatales	100%	0	0	5	0.010	56	0.135
60.	*Paraphaeosphaeria michotii* (Westend.) O.E. Erikss.	epiphyte, endophyte, latent plant pathogen	Pleosporales	99%	0	0	0	0	12	0.029
61.	***Penicillium chrysogenum*** Thom + *P. citrinum* Thom + *P. decumbens* Thom + *P. goetzii* J.D. Rogers, Frisvad, Houbraken & Samson + *P. olsonii* Bainier & Sartory + *P. roseopurpureum* Dierckx + *P. spinulosum* Thom + *Talaromyces marneffei* (Segretain, Capponi & Sureau) Samson, N. Yilmaz, Frisvad & Seifert + ***T. rugulosus*** (Thom) Samson, N. Yilmaz, Frisvad & Seifert	saprotroph	Eurotiales	99-100%	15249	9.744	1398	2.665	439	1.055
62.	*Phaeococcomyces eucalypti* Crous & R.G. Shivas	black yeast-like, epiphyte, endophyte, latent plant pathogen	Chaetothyriales	100%	0	0	0	0	10	0.024
63.	*Phialosimplex* sp.	animal pathogen	Eurotiales	98%	0	0	0	0	7	0.017
64.	*Phoma herbarum* Westend	plant pathogen	Pleosporales	100%	0	0	0	0	9	0.022
65.	Pleosporales		Pleosporales	100%	0	0	0	0	9	0.022
66.	*Pleurophoma ossicola* Crous, Krawczynski & H.-G. Wagner	epiphyte, endophyte, latent plant pathogen	Xylariales	100%	1	0.001	0	0	0	0
67.	*Pseudogymnoascus roseus* Raillo	saprotroph	Incertae sedis	100%	6	0.004	0	0	0	0

Table 1. Continued.

68.	*Pseudopithomyces chartarum* (Berk. & M.A. Curtis) Jin F. Li, Ariyaw. & K.D. Hyde	plant and animal pathogen, saprotroph, keratin degrader	Pleosporales	100%	1	0.001	21	0.040	228	0.548
69.	*Rhinocladiella atrovirens* Nannf.	plant pathogen, human pathogen	Chaetothyriales	100%	0	0	0	0	26	0.062
70.	Rhytismataceae	plant pathogen	Rhytismatales	100%	1	0.001	0	0	0	0
71.	Saccharomycetales		Saccharomycetales	100%	0	0	0	0	32	0.077
72.	*Sarocladium strictum* (W. Gams) Summerb.	saprotroph	Hypocreales	99%	1663	1.063	0	0	0	0
73.	*Scheffersomyces coipomensis* (C. Ramírez & A.E. González) H. Urbina & M. Blackw. + *S. ergatensis* (Santa María) H. Urbina & M. Blackw	white yeast-like, plant pathogen		100%	0	0	0	0	22	0.053
74.	*Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not.	on dung of herbivores	Sordariales	99%	12	0.008	27	0.051	1373	3.299
75.	*Sphaerulina pseudovirgaureae* Quaedvl., Verkley & Crous	plant pathogen	Mycosphaerellales	99%	0	0	0	0	2	0.005
76.	*Sporothrix inflata* de Hoog	saprotroph, human pathogen	Ophiostomatales	100%	0	0	0	0	6	0.014
77.	*Strasseria geniculata* (Berk. & Broome) Höhn.	plant pathogen	Incertae sedis	99%	22	0.014	139	0.265	3646	8.761
78.	*Striatibotrys rhabdosporus* L. Lombard & Crous	epiphyte, endophyte, latent plant pathogen	Hypocreales	99%	113	0.072	0	0	12	0.029
79.	*Sugiyamaella paludigena* (Golubev & Blagod.) H. Urbina & M. Blackw.	saprotroph, entomopathogenic	Saccharomycetales	99%	1	0.001	3	0.006	37	0.089
80.	*Sydowia polyspora* (Bref. & Tavel) E. Müll.	plant pathogen	Dothideales	99%	2	0.001	1	0.002	187	0.449
81.	*Taphrina carpini* (Rostr.) Johanson + *T. deformans* (Berk.) Tul. + *Taphrina* sp.	plant pathogen	Taphrinales	99%	10	0.006	1	0.002	28	0.067
82.	*Tetracladium* sp.	epiphyte, endophyte, latent plant pathogen	Helotiales	99%	6	0.004	0	0	0	0
83.	*Thelebolus ellipsoideus* Brumm. & de Hoog	on dung of herbivores	Thelebolales	99%	0	0	0	0	7	0.017
84.	*Thyronectria strobi* (Hirooka, Rossman & P. Chaverri) Jaklitsch & Voglmayr	plant pathogen	Incertae sedis	99%	0	0	7	0.013	158	0.380
85.	Trichocomaceae		Eurotiales	99%	0	0	0	0	43	0.103
86.	*Trichoderma longibrachiatum* Rifai + ***T. reesei*** E.G. Simmons + *T. semiorbis* (Berk.) Jaklitsch & Voglmayr	saprotroph	Hypocreales	99%	1169	0.747	5125	9.771	2701	6.490
87.	*Trichomonascus ciferrii* (M.T. Sm., Van der Walt & Johannsen) Kurtzman & Robnett	epiphyte, endophyte, latent plant pathogen, human pathogen	Saccharomycetales	99%	0	0	0	0	7	0.017

Table 1. Continued.

88.	*Truncatella spadicea* S.J. Lee & Crous	plant pathogen	Amphisphaeriales	99%	2	0.001	0	0	16	0.038
89.	*Volucrispora graminea* Ingold, P.J. McDougall & Dann	epiphyte, endophyte, latent plant pathogen	Incertae sedis	99%	0	0	0	0	11	0.026
	Basidiomycota				**1283**	**0.8198**	**136**	**0.259**	**4927**	**11.839**
1.	*Armillaria ostoyae* (Romagn.) Herink	plant pathogen	Agaricales	100%	1	0.001	4	0.008	195	0.469
2.	*Athelia acrospora* Jülich	facultative plant parasite, corticioid	Atheliales	100%	1	0.001	0	0	42	0.101
3.	*Baeospora myosura* (Fr.) Singer	mushroom species	Agaricales	99%	14	0.009	0	0	0	0
4.	*Bjerkandera adusta* (Willd.) P. Karst.	plant pathogen	Polyporales	99%	34	0.022	7	0.013	147	0.353
5.	*Bulleromyces albus* Boekhout & Á. Fonseca	yeast-like	Tremellales	99%	2	0.001	0	0	0	0
6.	*Calocybe graveolens* (Pers.) Singer	mushroom species	Agaricales	99%	2	0.001	0	0	0	0
7.	*Ceraceomyces serpens* (Tode) Ginns	corticioid or resupinate, *in the Red List of Threatened Species*	Amylocorticiales	100%	0	0	0	0	5	0.012
8.	Ceratobasidiaceae		Cantharellales	98%	0	0	0	0	49	0.118
9.	*Ceratobasidium* sp.	plant pathogen	Cantharellales	99%	92	0.059	11	0.021	73	0.175
10.	*Coprinellus disseminatus* (Pers.) J.E. Lange	mushroom species	Agaricales	100%	3	0.002	0	0	0	0
11.	*Cryptococcus tephrensis* Vishniac	yeast-like	Tremellales	100%	4	0.003	5	0.010	79	0.190
12.	Cystofilobasidiales	yeast-like	Cystofilobasidiales	99%	0	0	0	0	11	0.026
13.	*Cystofilobasidium macerans* Samp.	yeast-like	Cystofilobasidiales	99%	0	0	0	0	11	0.026
14.	*Daedaleopsis confragosa* (Bolton) J. Schröt.	plant pathogen	Polyporales	100%	9	0.006	0	0	0	0
15.	*Dioszegia buhagiarii* Á. Fonseca, J. Inácio & Spenc. -Mart. + *D. fristingensis* Á. Fonseca, J. Inácio & J.P. Samp.+ *D. hungarica* Zsolt	yeast-like	Tremellales	99-100%	51	0.033	0	0	21	0.050
16.	Entylomatales		Entylomatales	100%	5	0.003	6	0.011	44	0.106
17.	*Fibroporia vaillantii* (DC.) Parmasto	saprotroph	Polyporales	98%	0	0	0	0	411	0.988
18.	*Filobasidium chernovii* (Á. Fonseca, Scorzetti & Fell) Xin Zhan Liu, F.Y. Bai, J.Z. Groenew. & Boekhout + *F. floriforme* L.S. Olive + *F. wieringae* (Á. Fonseca, Scorzetti & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	yeast-like	Filobasidiales	99-100%	2	0.001	0	0	35	0.084
19.	*Flammulina velutipes* (Curtis) Singe	mushroom species	Agaricales	99%	0	0	0	0	7	0.017

Table 1. Continued.

20.	*Hapalopilus rutilans* (Pers.) Murrill	polypore taxon	Polyporales	100%	0	0	0	0	18	0.043
21.	*Heterobasidion annosum* (Fr.) Bref.	plant pathogen	Russulales	100%	1	0.001	0	0	102	0.245
22.	*Hyphodontia quercina* (Pers.) J. Erikss. + *Hyphodontia* sp.	corticioid or resupinate	Hymenochaetales	99%	8	0.005	0	0	0	0
23.	*Hypholoma acutum* (Sacc.) E. Horak	mushroom species	Agaricales	99%	0	0	0	0	10	0.024
24.	*Itersonilia pannonica* (Niwata, Tornai-Leh., T. Deák & Nakase) Xin Zhan Liu, F.Y. Bai, J.Z. Groenew. & Boekhout + *I. perplexans* Derx	plant pathogen	Cystofilobasidiales	99%	55	0.035	0	0	344	0.827
25.	*Leptosporomyces galzinii* (Bourdot) Jülich	corticioid or resupinate	Atheliales	100%	0	0	0	0	12	0.029
26.	*Leucopaxillus tricolor* (Peck) Kühner	mushroom species	Agaricales	100%	0	0	0	0	16	0.038
27.	*Leucosporidiella creatinivora* (Golubev) J.P. Samp.	yeast-like	Leucosporidiales	99%	4	0.003	0	0	0	0
28.	*Malassezia globosa* Midgley, E. Guého & J. Guillot + ***M. restricta*** E. Guého, J. Guillot & Midgley + *M. sympodialis* R.B. Simmons & E. Guého + *Malassezia* sp.	yeast-like, human and animal pathogen		100%	3	0.002	10	0.019	1063	2.554
29.	*Meripilus giganteus* (Pers.) P. Karst.	polypore taxon	Malasseziales	100%	0	0	0	0	137	0.329
30.	*Mycena aurantiomarginata* (Fr.) Quél. + *M. pura* (Pers.) P. Kumm.	mushroom species	Polyporales	99%	6	0.004	0	0	5	0.012
31.	*Naganishia adeliensis* (Scorzetti, I. Petrescu, Yarrow & Fell) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout + *N. diffluens* (Zach) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	yeast-like, human and animal pathogen	Tremellales	99%	122	0.078	2	0.004	45	0.108
32.	*Peniophora incarnata* (Pers.) P. Karst. + *P. pini* (Schleich. ex DC.) Boidin	plant pathogen	Russulales	99%	15	0.010	4	0.008	110	0.264
33.	*Phaeoclavulina flaccida* (Fr.) Giachini	coral taxon	Gomphales	99%	0	0	0	0	13	0.031
34.	*Phellinus pini* (Brot.) Pilát	plant pathogen	Hymenochaetales	99%	2	0.001	7	0.013	67	0.161
35.	*Phlebia radiata* Fr. + *Ph. tremellosa* (Schrad.) Nakasone & Burds.	facultative plant parasite, corticioid or resupinate	Polyporales	100%	9	0.006	0	0	0	0
36.	*Phlebiella christiansenii* (Parmasto) K.H. Larss. & Hjortstam	corticioid or resupinate	Polyporales	99%	0	0	0	0	18	0.043
37.	*Phlebiopsis gigantea* (Fr.) Jülich	saprotroph	Polyporales	100%	1	0.001	0	0	3	0.007
38.	*Puccinia caricina* DC.	plant pathogen	Pucciniales	100%	0	0	0	0	23	0.055
39.	*Resinicium bicolor* (Alb. & Schwein.) Parmasto	corticioid or resupinate	Incertae sedis	100%	7	0.004	27	0.051	451	1.084
40.	*Rhizoctonia solani* J.G. Kühn + *Rhizoctonia* sp.	plant pathogen	Cantharellales	100%	150	0.096	0	0	51	0.123

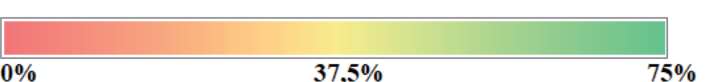
Table 1. Continued.

41.	*Rhodocollybia butyracea* (Bull.) Lennox	mushroom species	Agaricales	99%	10	0.006	0	0	0	0
42.	*Rhodosporidium kratochvilovae* Hamam., Sugiy. & Komag.	yeast-like	Sporidiobolales	100%	141	0.090	0	0	0	0
43.	*Rhodotorula mucilaginosa* (A. Jörg.) F.C. Harrison + *Rhodotorula* sp.	yeast-like, plant pathogen	Sporidiobolales	100%	331	0.212	1	0.002	18	0.043
44.	Russulales		Russulales	100%	4	0.003	4	0.008	94	0.226
45.	*Schizophyllum commune* Fr.	plant pathogen, polypore taxon	Agaricales	100%	5	0.003	0	0	0	0
46.	*Sebacina* sp.	coral taxon	Sebacinales	98%	0	0	0	0	59	0.142
47.	*Sistotrema* sp.	corticioid or resupinate	Cantharellales	100%	2	0.001	0	0	17	0.041
48.	*Sistotremastrum suecicum* Litsch. ex J. Erikss.	plant pathogen, corticioid or resupinate	Trechisporales	100%	0	0	24	0.046	30	0.072
49.	*Sporidiobolus metaroseus* J.P. Samp. & E. Valério + *S. pararoseus* Fell & Tallman + *S. salmonicolor* Fell & Tallman	yeast-like, plant pathogen	Sporidiobolales	100%	85	0.054	3	0.006	431	1.036
50.	*Sporobolomyces coprosmae* Hamam. & Nakase + *S. ruberrimus* Yamasaki & H. Fujii ex Fell, Pinel, Scorzetti, Statzell & Yarrow	yeast-like	Sporidiobolales	100%	4	0.003	0	0	9	0.022
51.	*Steccherinum ochraceum* (Pers.) Gray	facultative plant parasite, corticioid or resupinate	Polyporales	98%	0	0	0	0	13	0.031
52.	*Strobilurus albipilatus* (Peck) V.L. Wells & Kempton + *S. stephanocystis* (Kühner & Romagn. ex Hora) Singer	plant pathogen, mushroom species	Agaricales	99%	5	0.003	0	0	15	0.036
53.	*Symmetrospora coprosmae* (Hamam. & Nakase) Q.M. Wang, F.Y. Bai, M. Groenew. & Boekhout	yeast-like	Incertae sedis	100%	5	0.003	0	0	0	0
54.	*Tilletiopsis pallescens* Gokhale	mycoparasite	Incertae sedis	100%	10	0.006	0	0	53	0.127
55.	*Trametes gibbosa* (Pers.) Fr.+ *T. versicolor* (L.) Lloyd	saprotroph	Polyporales	100%	10	0.006	19	0.036	210	0.505
56.	Tremellales	plant pathogen	Tremellales	99-100%	1	0.001	0	0	11	0.026
57.	*Tritirachium oryzae* (Vincens) de Hoog	human and animal pathogen	Incertae sedis	99%	58	0.037	0	0	0	0
58.	*Vishniacozyma victoriae* (M.J. Montes, Belloch, Galiana, M.D. García, C. Andrés, S. Ferrer, Torr.-Rodr. & J. Guinea) Xin Zhan Liu, F.Y. Bai, M. Groenew. & Boekhout	saprotroph	Tremellales	100%	1	0.001	0	0	199	0.478
59.	*Wallemia muriae* (J. Kickx f.) Zalar & de Hoog	xerophilic	Wallemiales	99%	8	0.005	2	0.004	72	0.173

Table 1. Continued.

60.	*Xylodon flaviporus* (Berk. & M.A. Curtis ex Cooke) Riebesehl & Langer	corticioid or resupinate	Hymenochaetales	99%	0	0	0	0	78	0.187
	Myxomycota				**55**	**0.035**	**40**	**0.076**	**0**	**0**
1.	*Physarum loratum* Shuang L. Chen, Yu Li & H.Z. Li		Physarales	98%	55	0.035	40	0.076	0	0
	Plantae				**0**	**0.000**	**0**	**0.000**	**3**	**0.007**
1.	*Salvia syriaca* L.		Lamiales	100%	0	0.000	0	0.000	3	0.007
	Culturable fungi				**156393**	**99.935**	**52355**	**99.817**	**40689**	**97.775**
	Non-culturable fungi			97-100%	**9**	**0.006**	**37**	**0.071**	**548**	**1.317**
	Organisms with no reference sequence in NCBI				**37**	**0.024**	**19**	**0.036**	**375**	**0.901**
	Number of fungal taxa				**137**		78		170	
	Number of taxa				**142**		**88**		**205**	
	Margalef's diversity index-DMg				11.28		7.34		16.14	
	Shannon's diversity index-H				2.04		1.72		3.55	
	Simpson's diversity index–D				0.18		0.27		0.05	
	Shannon's evenness index-E				0.41		0.39		0.68	
	Berger–Parker's dominance index-d				0.27		0.39		0.15	
	Sorensen's qualitative similarity index-CN				0.57					
							0.44			

Sorensen's similarity index-CN for sample 1 and sample 3 was 0.56
Bold – the most abundant taxa



by 1, 8, 131 and 83 taxa, respectively. Non-culturable organisms were represented by 70 taxa. More than 90% of taxa were identified to genus or species. There were 142 and 205 taxa in parchment (samples 1 and 3) and 88 taxa in paper (sample 2). The number of species shared by parchment and paper from a single archive in Toruń was 9, and by the two parchments from two different archives (Toruń and Warsaw) was 33 (Fig. 1). The number of species shared by all three samples was 61, and the numbers of taxa separate for samples 1, 2 and 3 were respectively 36, 1 and 85.

Fungal communities consisted of the few abundant taxa and many rare taxa. The most frequent were Ascomycota from the genera *Acremonium*, *Alternaria*, *Aspergillus, Beauveria, Bionectria, Botrytis, Chaetomium, Penicillium* and *Trichoderma*. The most common were *Chaetomium murorum, Chaetomium globosum* and *Chaetomium iranianum.*

Communities included plant pathogens, epiphytes or endophytes of a wide range of plants, and possibly facultative plant pathogens, saprotrophs known from soil, decaying leaves, needles, wood (soft, white and brown rot fungi) and other plant material, human, animal and nematode pathogens, entomopathogenic taxa with worldwide distribution and a relatively wide host range, mycoparasites, white and black yeast-like taxa, taxa with medicinal properties, lichenized fungi, food contaminants, indoor contaminants, taxa known from herbivore dung, keratin-degrading taxa, mushroom-producing taxa, corticioid, resupinate, polypore, coral annual or perennial taxa, taxa with antagonistic properties, xerophilic taxa and endangered fungi included in the Red List of Threatened Species. The mycobiota of parchment, compared with that of paper, had: (i) more diversity, indicated by DMg (16.14 and 11.28 versus 7.34), H (3.55 and 2.04 versus 1.72) and D (0.05 and 0.18 versus 0.27); (ii) more even distribution of species, indicated by E (0.68 and 0.41 versus 0.39); and (iii) less dominance of single species, indicated by d (0.15 and 0.27 versus 0.39). There was more similarity in communities on paper and parchment stored in one place (Toruń) (CN = 0.57) and on parchment stored in different places (CN = 0.56) than on paper and parchment stored in two different places (CN = 0.44) (Table 1).

Discussion

Historical paper or parchment documents should be stored in a cool, stable environment – ideally at 20ºC and 50% relative humidity in a well-insulated and ventilated room, with no direct daylight or artificial light, in an acid- and lignin-free storage box. Such conditions had not always been provided for the documents studied. The torah, in particular, had been moved continually, used during religious ceremonies, and stored in the past in accidental places in non-controlled conditions. This may explain its advanced deterioration and high microbial contamination.

A non-destructive and non-invasive method for quantitative characterization of deterioration, based on spectral measurements, was applied to evaluate the scale of damage of the studied documents. The collagen-to-gelatin ratio, which can be estimated from the synchronous fluorescence spectra of modern and historical samples and of pure collagen and gelatin data, was applied for characterization of parchment condition. Analysis of fluorescence peaks and their comparison with peaks of other studies indicated the moderate stage of chemical deterioration of the studied documents. If there was at least 225 fungal taxa detected on both documents, their state seemed to result partly from fungal colonization.

The mycobiota of historical parchment documents has not received as much attention as the mycobiota of paper documents. Studies of parchment and leather have concentrated mostly on their physical damage and chemical changes and only rarely on their microbial colonization [1, 5, 7, 15, 16]. The greater diversity of mycobiota on parchment compared with paper (170 and 137 versus 87 taxa) supports earlier findings [6]. Studies emphasize a parchment's vulnerability to fungal infestation and colonization, resulting from its **c**hemical properties. Parchment contains proteins (~95% collagen) and some lipids (glycerides). With their low pH, both are ideal sources of nutrients for fungi. The preparation of parchment includes treatment with lime, which neutralizes the excessive acidity. Additional compounds (e.g., gum Arabic used for repair of document 1) might be additional sources of specific nutrients, increasing the spectrum of microorganisms.

The vulnerability of parchment to fungal infestation can also result from its extremely strong response to environmental variation. It deforms and deteriorates with the slightest change in temperature and humidity. Physical changes may be followed by chemical degradation; in high humidity the collagen fibres lose their initial high thermal and mechanical stability and convert to gelatin, which can easily be degraded by many fungal taxa, including *Alternaria alternata, Aspergillus* spp., *Mucor plumbeus*, *Penicillium chrysogenum, Phoma herbarum* and *Trichoderma longibrachiatum* detected by us and others [29, 30]. *Aspergillus versicolor* and *Penicillium chrysogenum* are among the most efficient, degrading 25-30% of gelatin in 2-3 weeks after colonization [16, 30].

The number of taxa presented here, detected with the Illumina technique applied, was much higher than in other studies, when only nine species from *Aspergillus*, *Mucor, Phoma* and *Penicillium* genera in nine historical parchment documents were detected with multiphasic approach applied, various sampling procedures and different microbiological methods [5], and 42 species (mostly from *Alternaria*, *Cladosporium*, *Epicoccum* and *Penicillium* genera) were detected with classical and molecular methods [15].

The most frequent taxa detected belonged to the Ascomycota. Often they are ubiquitous fungi present in human habitations due to intensive reproduction, easy dispersal, strong degrading properties and wide habitat range [5, 7, 10, 15, 16, 31, 32]. They usually have cellulolytic and proteolytic properties [5, 33-37]. Basidiomycota occurred more rarely. The proteolytic activity of Basidiomycota has been confirmed in the genera of *Armillaria, Cantharelus, Russula* and *Schizophyllum* [38]. The number and diversity of proteases produced by these taxa seem to be remarkable. Proteolysis by other taxa among those detected can also be expected, even though it has not yet been studied or proven. Production of proteases in Basidiomycota, however, seems to be regulated by the carbon and nitrogen sources and C:N ratio and activated at low levels of compounds containing accessible nitrogen [39, 40]. Parchment rich in nitrogen therefore may not be the best substrate for Basidiomycota.

Some taxa detected were more or less specialized. One of those is *Chaetomium*, which occurred in Document 1 with 53.12% and 73.07% frequency. The fungus is a specialist colonizer of leather and skin due to its utilization of collagen and keratin. Its dominance can also result from: (i) successful survival during long-distance travels, (ii) coping with extreme conditions at high elevations, (iii) resistance to antifungal substances produced by neighbours, and (iv) the production of antimicrobial compunds [41-43].

We recorded a few species of *Cladosporium*, but they were less frequent than in other studies. The most common *Penicillium* was *Penicillium chrysogenum*, which was also the predominant species on other parchment documents [5, 16]. Another *Penicillium*, i.e., *P. decumbens*, was common in one study [16] but rare in ours.

Acremonium charticola, Beauveria bassiana, Bionectria rossmaniae, Botrytis cinerea, Chaetomium murorum, Fusarium armeniacum, *Fusarium avenaceum*, *Fusarium sporotrichioides, Malassezia globosa, Mortierella* spp., *Sarocladium strictum*, *Serpula himantioides, Sordaria fimicola*, *Strasseria geniculata*, and most species of *Aspergillus* and *Penicillium* recorded had never been isolated previously from parchment documents. *Bjerkandera adusta* and *Phoma herbarum* were detected only recently [5].

Many of the species detected occur naturally as epiphytes on a wide range of plants. Some, however, are known from their extreme habitats. *Candida sake, Cystofilobasidium macerans, Leucosporidiella creatinivora, Mortierella* spp., *Naganishia adeliensis, Tetracladium, Thelebolus ellipsoideus* and *Volucrispora graminea* prefer moist and cold habitats [44]. *Exophiala xenobiotica* is known for habitats rich in monoaromatic hydrocarbons and alkanes. *Naganishia* spp. and *Wallemia* spp. are flexible 'opportunitrophs' known from the most extreme terrestrial habitats on Earth [45].

The group of the most unexpected and surprising taxa included: (i) *Arachniotus aurantiacus*, *Candida boleticola,* and *Debaryomyces prosopidis,* known from dung, mushrooms and exudates of mesquite trees, respectively; (ii) *Candida sake, Naganishia adeliensis,* and *Vishniacozyma victoriae,* known from seawater, decaying algae, mosses, lichens and soil in extremely cold, Arctic and Antarctic habitats [44, 46]; (iii) *Leptospora rubella* and *Phaeococcomyces eucalypti*, known from living leaves and leaf litter of *Eucalyptus*, and (iv) *Pleurophoma ossicola* and *Rhodotorula mucilaginosa,* known from bone and human beings [47]. The presence of taxa known from remote locations or non-native plants can be explained by fungal mobility. Microorganisms can be transported over long distances. Biogeographic patterns of microbial community structure show widespread long-distance dispersal on a global scale [48-50].

Some of the fungi detected may be potential opportunistic pathogens in immunocompromised patients. They may cause allergies, asthma, infections of the eye, ear, skin, nails, sinus, lungs, joints, bones, brain or the central nervous system [51-53]. Extracellular proteolytic enzymes may contribute to their pathogenicity on humans [33].

There is, however, an example of a beneficial, commensal human-fungus relationship involving *Malassezia globose,* which has been detected in both documents. Though the fungus seems to play a pathogenic role in several dermatologic conditions, its proteases may hydrolyse *Staphylococcus aureus* protein A – an important virulence factor involved in immunity evasion and infection on human skin [37].

Mycological analyses of archived documents often give different results depending on the character and age of the substrate, conditions of storage, inter-species relationships and interactions, and techniques used [2, 4, 7]. Older collections are generally colonized by higher numbers of fungi [15]. Exposure to light, suitable pH, emissions, pollutants including ozone, and volatile organic and inorganic compounds are vital [54]. Each document has its own history and its own specific contaminants. Each item should therefore be considered as a unique example with its own specific characteristics.

The culture-independent method used in this study contributes to a more complete survey of the mycobiota. This approach does not, however, give any information on the functional role of a particular taxon. The Illumina technique used based on rDNA gene detection is unable to give any information on the biodegradative potential of fungi detected and hazard connected with their presence in an archive. The molecular analysis used might have referred to DNA fragments that do not belong to microorganisms currently present. The method does not explain whether particular taxa can grow in parchment or occur only in the form of dormant propagules that fall onto the document from the air. Therefore, while the analysis has identified significant and potentially important diversity of fungi, it remains incomplete without information on their activity.

Analysis of both diversity and activity of fungi would be possible using transcriptomic technologies that would provide a broad account of active and dormant cellular processes.

Conclusions

Old written documents or other items can have priceless historical value to our cultural heritage, whether used for communication or religious purposes. Studies of biodeterioration-related fungi in documents and religious objects are important for understanding the scale of the biodeterioration process, and the necessity for and methods of conservation.

Acknowledgements

The authors are grateful to Prof. Elżbieta Jabłońska from the Department of Paper and Leather Conservation, Nicolaus Copernicus University in Toruń, Poland, for her kind collaboration.

Conflict of Interest

The authors declare no conflict of interest.

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