Journal of Scientific & Industrial Research
Vol. 83, September 2024, pp. 1012-1021
DOI: 10.56042/jsir.v83i9.7894

Amylolytic and Proteolytic Bacteria in Deteriorated Paper-based Historical Manuscripts

Yasemin Ünlü-Yokuş[1*], Duygu Göksay-Kadaifçiler[2] & Esra Ilhan-Sungur[2]

[1]Department of Biology, Institute of Graduate Studies in Sciences, [2]Department of Biology, Faculty of Science, Istanbul University, 34134, Vezneciler, Istanbul, Türkiye

Received 08 January 2024; revised 19 August 2024; accepted 28 August 2024

Amylolytic and proteolytic bacteria in the historical manuscripts can consume vegetable and animal based surface coating substances and adhesives which are found on the raw paper as a protective layer. Therefore, the presence of these bacteria in the historical manuscripts may pose a risk to the integrity of them. Moreover, the presence of bacteria showing enzymatic activities may cause a more serious threat to the deterioration of the historical manuscripts. In this study, it was mainly aimed to detect bacteria having all 3 enzymes together, amylase, caseinase and gelatinase, in the historical manuscripts. For this purpose, 10 historical manuscripts in the Süleymaniye Manuscript Library were screened for bacteria capable of producing amylase, caseinase and gelatinase enzymes and then the isolates found to produce all of these enzymes were identified by molecular methods. The obtained 85 bacterial isolates, 64%, 79%, and 53%, respectively, showed amylase, caseinase, gelatinase activity. Twenty-seven isolates (32%) were found to produce all of these 3 enzymes. Among these bacteria, amylase showed the highest hydrolysis ability in terms of enzyme index value. The bacteria were mainly included in the *Bacillus* genus belonging to the Firmicutes phylum (81%). The bacterial species with the highest hydrolysis zones for all 3 enzymes belonged to the genus *Bacillus*. The results may indicate that historical manuscripts using starch as surface coating and adhesive may be more susceptible to microbial damage, especially by the *Bacillus* genus.

Keywords: Amylolytic bacteria, Caseinolytic bacteria, Gelatinolytic bacteria, Historical manuscript, Microbial deterioration

Introduction

Libraries are environments rich in organic material that microorganisms can easily colonize and benefit. As a matter of fact, bacteria belonging to Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria phyla were reported in the historical manuscripts. These bacteria were predominantly *Bacillus*, *Stenotrophomonas*, *Staphylococcus* and *Microbacterium* species[1] and specialize in the production of hydrolytic enzymes. The release of hydrolytic extracellular enzymes, aggressive metabolic products, may increase material loss and create a significant threat to the deterioration of especially the historical manuscripts in libraries.[2] If proper storage conditions in terms of relative humidity and temperature are not provided for paper-based historical manuscripts, permanent deterioration may occur in the manuscripts caused by microorganisms. These damages can be observed in the forms of spots of different colors on the paper or a cottony appearance on the paper due to wear.

*Author for Correspondence
E-mail: yaseminunluyokus@gmail.com

Paper used in historical manuscripts (books, documents, prints, etc.) is a substrate usually obtained by pulping fibers of different origins and drying them in sheets, and mechanically processed using animal and vegetable adhesives, minerals and salts. The most common ingredient in paper, especially historical paper, is cellulose. Historical papers usually contain 90–99% cellulose fibers as a basic structural element. Cellulose consists of long linear chains of glucose. The polymer chains that make up cellulose are parallel to each other and form fibrils by connecting to each other through hydrogen bonds. Animal and vegetable-based surface coating substances (starch, egg yolk etc.) and adhesives (gelatin, casein, starch) were used in the production and/or restoration of historical papers. Among the most commonly used protein-based adhesives are gelatin and casein.

Some materials used in the production stage of paper such as surface coating substances, adhesives, ink etc. can be a source of nutrients for microorganisms.[3] Consumption of these substances by microorganisms can cause physical, chemical and aesthetic changes in historical manuscripts.[4] One of the microbial deterioration ways of historical papers is the consumption of animal and vegetable-based surface coating substances and adhesives as nutrients by microorganisms.[5] Starch is a mixture of two different polysaccharide fragments (amylose and amylopectin) obtained from plants and is hydrolyzed by the enzyme amylase. Hydrolysis of protein and polysaccharide-based adhesives by the proteolytic and amylolytic activities of microorganisms may cause deterioration in the integrity of manuscripts.[3] Starch is a homopolymer of glucose, like cellulose. However, starch consisting of amylose and amylopectin can be easily hydrolyzed by amylase, while hydrolysis of cellulose with crystalline structure is typically carried out by a cellulase complex with the participation of 3 different enzymes (endo-β-glucanase, exo-β-glucanase and β-glucosidase) and this structure is also highly resistant to hydrolysis by heterotrophs. Microorganisms prefer sugars that can be broken down more easily and quickly. Therefore, most of the bacteria such as *Bacillus*, *Pseudomonas* and *Clostridium* may exhibit the ability to produce amylase rather than cellulase. Amylase enzymes, which are known to show high stability over a wide range of pH and temperature, can quickly remove the adhesive properties of starch on the manuscript surfaces and lead to the deterioration of the integrity of the manuscript. Animal based surface coating substances and adhesives can be broken down by proteases. Proteases are classified according to their optimum pH and subtilisin is an alkaline protease that is frequently encountered in *Bacillus* species. Most alkaline proteases are non-specific and easily hydrolyzeany peptide bond. Protease enzymes can decrease the adhesive properties of protein-based materials on the manuscript surfaces and lead to the deterioration of the integrity of the manuscript.[2]

Since the main chemical component of paper is cellulose, it is assumed that cellulolytic species are typically responsible for the deterioration of paper. As a matter of fact, studies on the deterioration of the historical manuscripts generally focused on cellulolytic bacteria.[6,7] However, there is an important point that in order for microorganisms to reach the cellulose fibers of the paper, the surface coating (such as starch) and additives (casein, gelatin) on the upper layer of the paper must be hydrolyzed by amylolytic and proteolytic microorganisms. Therefore, the presence of amylolytic and proteolytic bacteria in the historical manuscripts may pose a serious risk to the integrity of them. Moreover, the consumption of these structural elements by these bacteria may facilitate cellulolytic bacteria to use cellulose. But it has been realized that studies on amylolytic and proteolytic bacteria in historical manuscripts are limited.

The Süleymaniye Manuscripts Library in Istanbul is an important library that contains historical manuscripts. Although, today, ideal conditions are provided in the new generation storage areas for the manuscripts in the library, there are some manuscripts damaged in the past due to many reasons (fire, flood, improper storage and transportation, etc.). These manuscripts may harbor bacteria to cause further damage by breaking down the vegetable (starch) and/or animal (gelatin and/or casein) adhesives as the protective layer of the raw paper in the manuscripts with their extracellular enzymes. Moreover, the presence of bacteria showing all of these enzymatic activities may pose a more serious threat to the deterioration of the historical manuscripts. Therefore, in this article, it was aimed to screen the amylase, gelatinase and caseinase enzymes of the isolates from historical manuscripts deteriorated in differently in the Süleymaniye Manuscripts Library, and to identify the bacterial isolates having all 3 enzyme activities together by molecular methods.

Materials and Methods

Sampling Procedure

Ten different biodeteriorated historical manuscripts (M1, M2, M3, M4, M5, M6, M7, M8, M9, and M10) were selected from the archive of the Süleymaniye Manuscript Library (Istanbul, Türkiye, according to observable biodeterioration signs, such as paper discoloration (in different shapes, colors, sizes), and texture changes (Fig. 1).

Samples from the deteriorated areas of each manuscript were collected with sterile nitrocellulose membrane filters (NCM) (Sartorius AG, Göttingen, Germany) a non-destructive sampling method. Firstly, 47 mm in diameter (corresponding to an area of 17.34 cm^2) -NCM filters were moistened with sterile PBS-Tween 20 (Sigma-Aldrich, MO, USA) and gently pressed on the surface of the manuscript for 30 s.[8] Then membrane filters were placed directly on culture plates in aseptic conditions. All samples were immediately transported to the laboratory. During the sampling process, the temperature, and the relative humidity of the storage room of historical manuscripts were recorded as 18°C and 50%, respectively.

Fig. 1 — Historical manuscripts from which samples were taken

Bacterial Isolation

Reasoner's 2A (R2A) medium was used for isolation of aerobic mesophilic heterotrophic bacteria (AMHB) from the biodeteriorated historical manuscripts. NCM filters sampled were placed directly onto R2A under sterile condition and incubated at 28°C for 7 days.[9] The inoculations were performed in triplicate and all media contained cycloheximide (50 mg·mL^{-1}) to inhibit fungal growth. After incubation, all isolates were screened through colony morphology, Gram staining and microscopic morphology. Pure cultures of the isolates were obtained by sub-culturing in R2A and maintained on R2A slants at 4°C for further studies and stored in glycerol suspension for bio-conservation.

Extracellular Enzymes Analysis

The bacterial isolates were inoculated in Luria-Bertani (LB) broth. After incubation overnight at 28°C, 5 μL of fresh bacterial culture was inoculated on enzyme-specific media to detect extracellular enzymatic activity for amylase, caseinase, and gelatinase.

In order to screen for amylolytic activity, the bacterial isolates were cultured on starch agar (SA). After incubation at 37°C for 24 hours, the medium was stained with Gram's iodine. The presence of amylase-producing isolates was detected with the formation of a clear zone around the colony.[10-12]

In order to determine the proteolytic activities of bacteria, the abilities to produce both caseinase and gelatinase enzymes were investigated. The bacterial isolates were cultivated on skim milk agar (SM-agar) and gelatin agar (GA) to detect caseinase and gelatinase activities, respectively.[13] SM-agar and GA plates were incubated at 30°C for 4 day and 28°C for 48 hours, respectively. Proteolytic species were determined by the formation of a clear zone around the colony at the end of incubation periods.[9,14]

All tests were performed in triplicate. Photographs of bacterial isolates showing enzymatic index values were taken, colony diameters and zone diameters were measured using the ImageJ program and the enzymatic index was calculated using the formula below:[15]

$$Enzymatic\ index = \frac{colony\ diameter + zone\ diameter}{colony\ diameter}$$

Molecular Identification

For molecular identification, the bacterial isolates that were capable of synthesizing all of 3 enzymes (amylase, caseinase, and gelatinase) were selected.

The isolates were inoculated in fresh LB Broth and incubated at 28°C for 24 h. Genomic DNA was extracted from the cell pellet using a PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) as described by the manufacturer. The quality of the extracted DNA samples was checked on 1.0% agarose gel (Invitrogen, Carlsbad, CA, USA) stained with GelRed (Biotium, Hayward, CA. USA). All genomic DNA samples were stored at –20°C for further processes.

Bacterial 16S rDNA gene fragments were amplified from the genomic DNA of pure bacterial isolates using universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1495R (5'-CGGCTACCTTGTTACGACTT-3').[16,17] PCR amplifications were performed in 50 μL mixtures containing 1 μL bacterial genomic DNA, 0.76 μLof each primer, 22.48 μL nuclease-free water (Qiagen, Germany), 25.0 μL Taq Master Mix (Qiagen, Germany), and thermocycling was performed in a T100 thermal cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) under the following conditions: initial denaturation at 95°C for 5 min, 34 amplification cycles (denaturation at 95°C for 5 s, annealing at 59°C for 60 s, and an elongation step at 72°C for 105 s), and final elongation step at 72°C for 10 min.The quality of the PCR products was checked on 1.0% agarose gel (Invitrogen, Carlsbad, CA, USA) stained with GelRed (Biotium, Hayward, CA. USA).

The PCR products were purified and sequenced by a commercial company (Eurofins Genomics, Constance, Germany). The determined DNA sequences were aligned by Clustal X software.[18] The resulting multiple sequence alignment was edited by GeneDoc software.[19] Afterwards, the consensus sequences obtained from the both strands were compared with the sequences stored in the GenBank database using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST®) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).[20] Sequence similarity threshold values, currently proposed to classify bacterial isolates, were considered.[21]

Statistical Analysis

SPSS 20.0 program was used to determine the normality of the distribution of the enzymatic index values and the Kruskal-Wallis test was used to determine whether there was a significant difference between the enzymatic index values of 3 independent enzymes.

Results and Discussion

The Isolates

Eighty-five bacterial isolates differing in terms of macroscopic and microscopic properties were obtained from the samples. Ninety-two point nine percent of these isolates are Gram positive. Most of these isolates (74.1%) had bacillus shape, while the others were in coccus (21.1%) and coccobacillus (4.8%) morphology. Bahjat and Younis-Sherif reported that 46 of 49 bacteria isolated from historical documents were Gram positive and had bacillus morphology. Gram positive bacteria have a cell wall consisting of a thick layer of peptidoglycan, which makes them resistant to harsh physicochemical conditions.[22] In addition, some Gram positive bacillus shaped bacteria, such as *Bacillus*, can survive much longer than vegetative cells by forming endospores under harsh environmental conditions.[23] Also, Gram positive bacteria have a high potential to degrade various biopolymers.[24] For these reasons, Gram positive bacteria may be widely isolated from organic matter-rich extreme habitats.

Extracellular Enzymatic Profiles of the Isolates

The ability of 85 isolates to produce amylase, caseinase, and gelatinase enzymes, which are supposed to be effective enzymes that contribute to the deterioration of manuscripts, were investigated.[3] Eighty-two (96%) of the bacterial isolates showed extracellular enzymatic activity. Similarly, Krakova *et al.*[25] reported that all 16 bacteria isolated from historical paper samples showed hydrolytic enzyme activity. However, Raeisnia *et al.*[26] estimated that 64% of the bacteria isolated from an 11th century manuscript produced extracellular enzymes.

While 32% (27 isolates) of the isolates had the ability to produce all of 3 enzymes (amylase, caseinase, and gelatinase), the number of isolates showing any 2 of the enzyme activities was 35% (30 isolates) (Fig. 2a–2b). Many bacteria were able to produce more than one enzyme.[26,27] The co-operation of more than one enzyme can facilitate the hydrolysis process[28] and can lead to accelerate the process of manuscript degradation. Therefore, the detection of bacteria capable of producing more than one hydrolytic enzyme is important for future studies in terms of taking prevention to slow down the biodegradation of manuscripts. Also, the number of isolates showing only one enzymatic activity was 29% (25 isolates) (Fig. 2). In addition, the most synthesized enzyme by the isolates was caseinase (79%), followed by amylase (64%) and gelatinase (53%) was detected. Alves *et al*. also reported the same results which caseinase.[27]

The amylolytic, caseinolytic, and gelatinolytic agar assays of 85 bacterial isolates are given in Table 1. Enzymatic index values of the bacterial isolates in SA, SM-Agar, and GA were found to be in the range

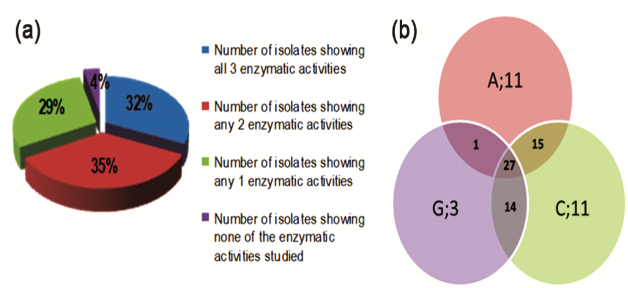

Fig. 2 — The (a) percentage and (b) the numerical representations of enzymatic profiles of bacterial isolates; (A) amylase, (C) caseinase and (G) gelatinase enzymes

Table 1 — The hydrolytic abilities of eighty-five bacterial isolates

Isolate code	Sample code	Starch agar ZD (mm)	Starch agar CD (mm)	Amylase EI	Skim milk agar ZD (mm)	Skim milk agar CD (mm)	Caseinase EI	Gelatine agar ZD (mm)	Gelatine agar CD (mm)	Gelatinase EI
S1	M1	16.42 ± 1.13	8.51 ± 1.65	1.98	23.78 ± 1.25	13.34 ± 0.91	1.78	21.14 ± 0.78	7.33 ± 0.69	2.90
S2	M1	10.64 ± 0.75	5.33 ± 0.36	2.00	22.43 ± 1.43	12.52 ± 0.29	1.79	30.14 ± 0.28	15.88 ± 3.33	1.96
S3	M1	21.54 ± 2.25	6.16 ± 0.97	3.53	23.34 ± 1.02	11.25 ± 0.79	2.08	22.76 ± 2.14	11.85 ± 2.05	1.94
S4	M3	24.10 ± 0.33	7.74 ± 0.66	3.13	41.50 ± 4.20	34.12 ± 2.56	1.22	22.30 ± 1.44	9.44 ± 1.09	2.38
S5	M3	12.79 ± 1.20	6.24 ± 0.68	2.08	21.35 ± 0.47	8.98 ± 0.26	2.38	18.38 ± 1.81	9.42 ± 0.54	1.95
S6	M3	20.59 ± 2.27	6.25 ± 0.62	3.30	37.53 ± 2.05	26.12 ± 2.82	1.45	24.15 ± 0.61	7.78 ± 0.58	3.11
S7	M4	20.29 ± 0.84	6.54 ± 0.16	3.10	15.36 ± 0.82	11.34 ± 0.68	1.36	12.26 ± 0.47	7.23 ± 0.26	1.70
S8	M4	13.09 ± 1.13	7.31 ± 0.80	1.81	21.54 ± 1.69	13.24 ± 1.00	1.64	24.56 ± 2.10	9.86 ± 0.77	2.51
S9	M4	31.06 ± 1.30	6.28 ± 0.36	4.96	19.42 ± 0.70	9.81 ± 0.20	1.98	15.15 ± 1.54	7.73 ± 0.31	1.97
S10	M5	19.07 ± 0.61	7.77 ± 0.85	2.47	28.48 ± 1.21	15.07 ± 0.71	1.89	22.60 ± 2.40	10.10 ± 0.64	2.24
S11	M8	22.04 ± 0.56	8.59 ± 0.57	2.58	35.24 ± 4.19	16.12 ± 3.35	2.21	25.18 ± 1.36	9.45 ± 0.85	2.67
S12	M10	20.24 ± 2.21	5.20 ± 0.50	3.89	24.29 ± 2.25	15.26 ± 0.77	1.59	20.21 ± 0.64	7.04 ± 0.74	2.90
S13	M5	15.61 ± 0.94	5.93 ± 0.34	2.63	27.40 ± 1.08	24.50 ± 1.80	1.12	25.65 ± 1.01	9.61 ± 0.58	2.68
S14	M5	25.26 ± 0.46	5.99 ± 0.49	4.24	30.00 ± 0.11	15.06 ± 1.37	2.00	21.15 ± 0.67	10.12 ± 0.13	2.09
S15	M9	22.07 ± 0.50	6.23 ± 0.18	3.55	25.82 ± 1.05	12.03 ± 1.03	2.16	23.03 ± 0.12	8.98 ± 0.53	2.57
S16	M10	23.69 ± 0.51	6.53 ± 0.42	3.63	29.21 ± 1.09	13.92 ± 0.51	2.10	27.43 ± 0.21	10.32 ± 0.33	2.66
S17	M1	26.43 ± 1.34	10.44 ± 0.29	2.53	27.72 ± 0.77	20.94 ± 0.60	1.32	—	—	—
S18	M1	12.12 ± 2.91	4.79 ± 0.63	2.53	—	—	—	—	—	—
S19	M2	15.59 ± 0.56	7.87 ± 1.15	2.00	33.05 ± 6.94	20.66 ± 5.70	1.62	—	—	—
S20	M2	15.44 ± 0.62	9.69 ± 0.38	1.60	31.18 ± 2.80	20.37 ± 4.59	1.57	—	—	—
S21	M2	35.89 ± 0.67	16.98 ± 0.22	2.11	54.97 ± 2.84	16.50 ± 0.74	3.34	32.69 ± 0.79	11.22 ± 0.31	2.92
S22	M2	32.68 ± 0.24	6.93 ± 0.38	4.72	35.11 ± 0.20	11.24 ± 0.61	3.13	29.14 ± 0.66	7.86 ± 0.17	3.71
S23	M2	19.58 ± 0.09	9.46 ± 0.39	2.07	30.14 ± 2.55	15.39 ± 1.16	1.96	—	—	—
S24	M2	13.64 ± 0.31	6.32 ± 0.21	2.16	29.29 ± 10.46	13.80 ± 2.11	2.09	—	—	—
S25	M2	15.22 ± 0.69	7.72 ± 0.82	1.98	30.09 ± 5.63	17.28 ± 2.96	1.74	—	—	—
S26	M3	—	—	—	29.59 ± 0.50	18.30 ± 1.46	1.62	—	—	—
S27	M4	46.06 ± 1.57	7.84 ± 0.08	5.87	26.22 ± 7.34	12.58 ± 3.41	2.20	—	—	—
S28	M4	34.00 ± 0.86	17.19 ± 0.86	1.98	25.08 ± 0.23	14.98 ± 2.46	1.70	—	—	—
S29	M4	26.62 ± 0.43	10.03 ± 0.47	2.66	23.41 ± 1.34	15.88 ± 0.38	1.47	—	—	—
S30	M5	47.13 ± 2.59	13.60 ± 0.45	3.47	43.40 ± 4.82	34.00 ± 2.76	1.29	32.91 ± 0.68	24.41 ± 0.45	1.35
S31	M5	16.59 ± 0.66	9.12 ± 0.09	1.82	20.75 ± 1.83	15.14 ± 0.39	1.37	—	—	—
S32	M5	15.04 ± 0.81	5.67 ± 0.32	2.66	21.11 ± 1.44	8.83 ± 0.48	2.39	—	—	—
S33	M7	24.47 ± 5.05	10.26 ± 2.36	2.40	18.04 ± 0.57	10.49 ± 0.50	1.72	—	—	—
S34	M6	42.88 ± 1.66	11.64 ± 0.55	3.69	54.29 ± 4.88	27.53 ± 0.83	1.97	34.17 ± 0.40	15.56 ± 1.31	2.20
S35	M8	—	—	—	—	—	—	—	—	—
S36	M8	18.66 ± 0.85	5.87 ± 0.05	3.18	—	—	—	—	—	—
S37	M8	31.17 ± 1.38	15.88 ± 0.24	1.96	—	—	—	35.61 ± 1.65	25.66 ± 1.05	1.39
S38	M8	43.11 ± 1.28	13.05 ± 0.42	3.31	19.94 ± 0.77	18.19 ± 1.04	1.10	32.94 ± 1.09	15.78 ± 0.95	2.09
S39	M8	41.22 ± 1.99	11.76 ± 0.67	3.52	22.31 ± 1.60	14.24 ± 0.75	1.57	20.91 ± 1.06	12.36 ± 0.53	1.69
S40	M9	—	—	—	37.73 ± 2.14	8.64 ± 0.16	4.37	—	—	—
S41	M10	—	—	—	40.27 ± 1.93	13.30 ± 0.53	3.03	20.52 ± 0.93	7.98 ± 0.36	2.57
S42	M10	30.75 ± 0.20	17.28 ± 0.97	1.78	35.65 ± 1.07	18.12 ± 0.79	1.97	27.70 ± 1.14	15.05 ± 0.81	1.84
S43	M10	—	—	—	24.79 ± 0.76	11.70 ± 0.15	2.12	21.58 ± 0.36	9.66 ± 0.72	2.24
S44	M10	33.34 ± 0.63	17.23 ± 0.41	1.94	41.70 ± 0.48	19.12 ± 0.61	2.18	27.53 ± 0.14	15.78 ± 0.43	1.75
S45	M4	12.89 ± 0.62	7.41 ± 0.30	1.74	27.65 ± 0.62	14.26 ± 0.42	1.94	16.57 ± 1.82	8.27 ± 0.56	2.00
S46	M1	30.76 ± 4.03	7.94 ± 0.24	3.87	45.50 ± 3.35	29.75 ± 1.18	1.53	14.36 ± 2.14	8.42 ± 0.85	1.70
S47	M5	26.08 ± 2.94	6.99 ± 0.43	3.76	42.68 ± 1.50	22.77 ± 0.31	1.87	25.38 ± 1.67	15.24 ± 0.78	1.67
S48	M1	—	—	—	17.33 ± 0.44	10.65 ± 0.71	1.63	—	—	—
S49	M1	—	—	—	29.08 ± 0.47	9.63 ± 0.24	3.02	23.92 ± 0.82	7.89 ± 0.33	3.03
S50	M2	—	—	—	28.87 ± 1.04	21.33 ± 0.50	1.35	24.57 ± 1.91	13.73 ± 5.38	1.95
S51	M3	—	—	—	28.71 ± 1.82	21.27 ± 1.29	1.36	—	—	—
S52	M3	—	—	—	28.71 ± 1.17	26.42 ± 1.11	1.09	18.45 ± 1.69	10.71 ± 0.85	1.73

(*contd.*)

Table 1 — The hydrolytic abilities of eighty-five bacterial isolates (*contd.*)

Isolate code	Sample code	Starch agar			Skim milk agar			Gelatine agar		
		ZD (mm)	CD (mm)	Amylase EI	ZD (mm)	CD (mm)	Caseinase EI	ZD (mm)	CD (mm)	Gelatinase EI
S53	M4	—	—	—	38.39 ± 0.39	16.80 ± 0.93	2.29	23.81 ± 1.83	10.93 ± 0.46	2.18
S54	M4	–	–	–	36.29 ± 1.30	12.43 ± 0.99	2.93	13.42 ± 0.69	6.38 ± 0.46	2.12
S55	M4	—	—	—	34.32 ± 1.95	10.81 ± 0.49	3.18	28.56 ± 1.39	8.93 ± 0.58	3.20
S56	M4	—	—	—	23.49 ± 3.44	11.98 ± 0.23	1.96	13.66 ± 1.95	6.47 ± 0.33	2.11
S57	M4	—	—	—	28.29 ± 1.72	15.02 ± 4.28	1.98	23.15 ± 0.95	8.76 ± 0.20	2.64
S58	M4	—	—	—	—	—	—	—	—	—
S59	M5	—	—	—	36.13 ± 1.90	14.72 ± 0.90	2.46	26.71 ± 0.77	8.70 ± 0.35	3.07
S60	M5	—	—	—	—	—	—	24.59 ± 0.98	9.69 ± 0.42	2.54
S61	M5	—	—	—	36.33 ± 0.67	14.98 ± 0.50	2.43	28.21 ± 0.52	10.75 ± 0.24	2.63
S62	M5	26.86 ± 0.80	12.81 ± 1.32	2.11	—	—	—	—	—	—
S63	M5	—	—	—	16.18 ± 0.92	14.64 ± 0.81	1.11	—	—	—
S64	M5	46.17 ± 2.78	16.32 ± 1.54	2.83	46.84 ± 1.23	28.16 ± 0.61	1.66	—	—	—
S65	M5	12.58 ± 0.54	8.05 ± 0.31	1.56	—	—	—	—	—	—
S66	M6	—	—	—	32.00 ± 2.58	13.55 ± 0.55	2.36	—	—	—
S67	M6	—	—	—	39.20 ± 1.32	11.80 ± 0.68	3.33	33.50 ± 2.31	8.74 ± 0.22	3.83
S68	M7	—	—	—	—	—	—	20.83 ± 1.95	13.52 ± 1.23	1.55
S69	M7	21.52 ± 0.68	9.88 ± 1.41	2.20	—	—	—	–	–	–
S70	M7	—	—	—	—	—	—	—	—	—
S71	M8	—	—	—	6.26 ± 0.23	3.01 ± 0.18	2.09	—	—	—
S72	M8	20.01 ± 0.10	17.35 ± 0.16	1.15	—	—	—	—	—	—
S73	M8	14.11 ± 0.45	7.66 ± 0.68	1.85	—	—	—	—	—	—
S74	M8	42.30 ± 1.80	15.36 ± 0.13	2.75	55.07 ± 3.10	27.37 ± 0.59	2.01	—	—	—
S75	M9	—	—	—	37.20 ± 3.26	14.02 ± 0.32	2.66	—	—	—
S76	M9	31.59 ± 2.01	11.20 ± 0.84	2.84	—	—	—	—	—	—
S77	M9	20.32 ± 0.84	10.80 ± 0.32	1.88	—	—	—	—	—	—
S78	M9	21.12 ± 0.13	8.35 ± 0.53	2.54	—	—	—	—	—	—
S79	M9	26.85 ± 0.53	10.36 ± 1.35	2.62	40.24 ± 1.72	21.63 ± 0.05	1.86	—	—	—
S80	M9	20.86 ± 0.88	7.94 ± 1.10	2.65	—	—	—	—	—	—
S81	M9	—	—	—	—	—	—	5.62 ± 0.97	2.97 ± 0.55	1.96
S82	M10	—	—	—	19.58 ± 0.59	10.32 ± 0.32	1.90	—	—	—
S83	M10	—	—	—	47.56 ± 1.14	30.95 ± 2.03	1.54	—	—	—
S84	M10	—	—	—	43.65 ± 1.04	17.24 ± 0.22	2.53	33.47 ± 0.41	12.45 ± 1.04	2.70
S85	M10	—	—	—	37.23 ± 3.74	9.23 ± 0.52	4.04	—	—	—

CD: Colony diameter (mm)
ZD: Zone diameter (mm) (Colony diameter + hydrolysis zone)
EI: Enzymatic index
—: Zone formation did not occur

of 4.96–1.60, 4.37–1.54, and 3.83–1.35, respectively. The hydrolysis abilities of the bacterial isolates in this study were higher than those in other few studies.[26,29] According to the results of the statistical analysis performed among 27 bacteria showing all of 3 enzymes, it was determined that there was a significant difference between amylase, caseinase, and gelatinase index values. Amylase index values were found to be significantly higher than caseinase and gelatinase index values ($p<0.01$). The results may indicate that historical manuscripts that use starch as a surface coating and adhesive may be at greater risk of microbial damage. Therefore, future studies are needed to determine the biodegradative effect of each enzyme of bacteria that can produce more than one hydrolytic enzyme on manuscripts.

Molecular Identification of the Isolates

Since enzymatic activity results were used as a preliminary evaluation criterion in the selection of bacterial isolates for molecular identification, 27 isolates capable of synthesizing all of 3 enzymes were identified. The 3 isolates (S45, S46 and S47) were unidentified bacteria (11%) (Fig. 3). The 22 bacteria (81%) were included in Firmicutes phylum (Table 2). Additionally, 1 bacterium (4%) included in the Proteobacteria and 1 bacterium (4%) Bacteroidetes phyla were also found in the manuscripts (Fig. 3).

Some studies on paper-based manuscripts were reported that bacteria in the Firmicutes phylum were predominant, while Bacteroidetes and Proteobacteria members were in less amount.[1,25,30]

It was determined that 19 of 24 bacterial isolates (S4, S5, S6, S7, S8, S9, S11, S12, S13, S14, S15, S16, S21, S22, S30, S34, S38, S42 and S44) belonged to *Bacillus* genus in Bacilli class of Firmicutes phylum with varying similarity rates (97–99%) (Table 2). Savoldelli *et al.*[31] similarly stated that the most detected bacterial genus in the historical documents was *Bacillus*. Also, *Bacillus* genus bacteria were frequently isolated from wall paintings, paintings, paper-based works, etc.[1,9,25,30] The fact that these bacteria are common in historical documents as well as in nature may be due to forming endospores, having extracellular enzyme diversity and using different catabolic reactions to obtain energy. It was assumed that they are the dominant bacterial genus in the historical documents, since they can survive for many years with their spore form.[32]

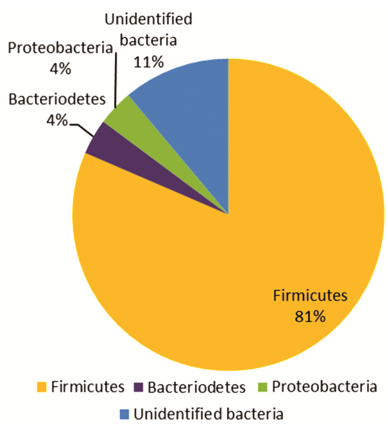

Fig. 3 — Phylogenetic distribution of the amylolytic, caseinolytic, and gelatinolytic bacteria obtained from historical manuscripts at phylum level

It was detected that the S4 and S5 isolates were related (99%) with *Bacillus halotolerans* and *Bacillus simplex,* respectively. These bacteria were determined in the historical documents.[33,34] The S6 isolate shared 97% identity with *Bacillus siamensis.* The S11 and S44 isolates showed identity (98% and 99%, respectively) to *Bacillus tequilensis*. Gatson *et al.*[35]

Table 2 — Phylogenetic affiliations on 16S rRNA analysis of determined bacterial isolates

Sample code	Isolate no	Closest BLAST® match and BLAST® accession number	Similarity (%)	Phylum
M1	S1	*Priestia aryabhattai* EAF36 (MG593997)	98	Firmicutes
	S2	*Priestia aryabhattai* OA091 (KY622738)	98	Firmicutes
	S3	*Chryseobacterium* sp. PCH55 (KY628873)	98	Bacteroidetes
	S46	NA*		
M2	S21	*Bacillus subtilis* JCM 1465 (NR_113265)	99	Firmicutes
	S22	*Bacillus subtilis* JCM 1465 (NR_113265)	99	Firmicutes
M3	S4	*Bacillus halotolerans* SR36 (MH010392)	99	Firmicutes
	S5	*Bacillus simplex* BD17-S24 (HF584831)	99	Firmicutes
	S6	*Bacillus siamensis LA084 (KY622204)*	97	Firmicutes
M4	S7	*Bacillus* sp. P5-2 (MG827115)	99	Firmicutes
	S8	*Bacillus* sp. JZHS21 (DQ658962)	98	Firmicutes
	S9	*Bacillus* sp. JZHS21 (DQ658962)	99	Firmicutes
	S45	NA*		
M5	S10	*Priestia aryabhattai* SKC15 (MF000970)	98	Firmicutes
	S13	*Bacillus subtilis* HHT-10 (HQ268531)	98	Firmicutes
	S14	*Bacillus mojavensis* HQB1354 (MH041231)	99	Firmicutes
	S30	*Bacillus wiedmannii* FSL W8-0169 (NR_152692)	99	Firmicutes
	S47	NA*		
M6	S34	*Bacillus mobilis* MCCC 1A05942 (NR_157731)	99	Firmicutes
M8	S11	*Bacillus tequilensis* JW18 (KP126484)	98	Firmicutes
	S38	*Bacillus mobilis* MCCC 1A05942 (NR_157731)	99	Firmicutes
	S39	*Pseudomonas chengduensis* MBR (NR_125523)	99	Proteobacteria
M9	S15	*Bacillus subtilis* HC-2 (KP835201)	99	Firmicutes
M10	S12	*Bacillus mojavensis* HQB1354 (MH041231)	98	Firmicutes
	S16	*Bacillus gaemokensis* KK16B1_9 (KY344805)	97	Firmicutes
	S42	*Bacillus subtilis* JCM 1465 (NR_113265)	99	Firmicutes
	S44	*Bacillus tequilensis* 10b (NR_104919)	99	Firmicutes

NA*: The species could not be determined.

isolated *B. tequilensis* from a 2000-year-old Mexican shaft-tomb and reported that it was genetically closely related to *Bacillus subtilis.* The S13, S15, S21, S22 and S42 isolates showed similarity (98–99%) with *B. subtilis*. It was noticed that *B. subtilis* bacteria were found in 4 of the 10 historical manuscripts studied. *B. subtilis* was also reported in archaeological manuscripts, historical photographs, parchment manuscripts, historical monuments and indoor air of the library.[6,36,37] *B. subtilis* was found to be the bacterium with the highest casein hydrolysis ability in the manuscripts.

The isolates S12 and S14 showed identity (98% and 99%, respectively) with *Bacillus mojavensis* which was detected in an 11th century manuscript.[26] The S16 and S30 isolates were related (97% and 99%, respectively) to *Bacillus gaemokensis* and *Bacilus wiedmanni*, respectively. The S34 and S38 isolates showed similarity (99%) with *Bacillus mobilis*. *B. gaemokensis, B. wiedmanni* and *B. mobilis* species were reported as members of the *Bacillus cereus* group.[38,39] *Bacillus* species adapt to microclimate conditions of low temperature and high humidity faster than other bacterial groups.[39] Among the *Bacillus* isolates, *B. wiedmanni* and *B. mobilis* species were found to have the highest hydrolysis ability of starch and gelatin, respectively.

The S1, S2 and S10 isolates showed 98% identity with *Priestia aryabhattai,* which is formerly known as *Bacillus aryabhattai* and was reported to be isolated from ancient Ola leaf manuscripts and the surface of the skeletons exhibited in a museum.[40,41]

The S3 isolate was closely related (99%) with the *Chryseobacterium* genus included in the Flavobacteria class of the Bacteroidetes phylum. *Chryseobacterium* species, which are non-fermentative Gram negative rod types with strong proteolytic activity, capable of oxidizing many carbohydrates, are widely found in soil and water.[42] Although *Chryseobacterium* species are known as rare human pathogens, they have been reported to cause bacteremia, pneumonia, meningitis, pyomyositis, and keratitis in hospital settings.[43]

The S39 isolate shared 99% identity with *Pseudomonas chengduensis,* which belonged to the Proteobacteria phylum, Gammaproteobacteria class and *Pseudomonas* genus. *Pseudomonas* species are Gram negative bacteria with opportunistic pathogenic properties that can be found widely in nature with different physiological characteristics. *Chryseobacterium* and *Pseudomonas* species have also been detected in deteriorated library materials and manuscripts.[25,31] *P. chengduensis* was found to be able to hydrolyze casein and starch unlike the study of Tao *et al.*[45] Since opportunistic pathogenic bacteria can be found in the historical manuscripts, library staff and visitors should contact the historical manuscripts with gloves.

Conclusions

The data on the metabolic capacities of microorganisms involved in the deterioration of historical manuscripts could offer useful for their preservation and conservation. The findings of this study may serve as a foundation for experiments aimed at eliminating bacteria that produce three enzymes (amylase, caseinase, and gelatinase) potentially threatening manuscript integrity.

The count of caseinolytic bacteria is higher than that of amylolytic and gelatinolytic bacteria, but amylolytic bacteria have a stronger hydrolysis ability. This suggests that historical manuscripts with starch-based surface coatings and adhesives might be at greater risk of bacterial damage. *Bacillus* bacteria, due to their prevalence and high hydrolysis capability, could significantly contribute to manuscript deterioration. Therefore, developing strategies to prevent *Bacillus* colonization might be beneficial for manuscripts preservation. Additionally, future research could focus on bacteria-specific disinfection treatment.

Acknowledgement

This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University. Project numbers are 21940 and 35646. The authors thank the Süleymaniye Manuscript Library Directorate and its staff for providing the necessary permissions and assistance during sampling.

Conflict of Interest

Authors declare no competing interests.

References

1 Okpalanozie O E, Adebusoye S A, Troiano F, Catto C, Ilori M O & Cappitelli F, Assessment of indoor air environment of a Nigerian museum library and its biodeteriorated books using culture-dependent and-independent techniques, *Int Biodeterior Biodegradation*, **132** (2018) 139–149, https://doi.org/10.1016/j.ibiod.2018.03.003.

2 Abdel-Maksoud G, Nasr H, Samaha S H & Kassem M S E, Analytical methods for evaluating the state of preservation of

a historical manuscript dating back to the 15th century AD in Al-Azhar Library–Egypt, *Pigm Resin Technol*, (2023).

3 Mazzoli R, Giuffrida M G & Pessione E, Back to the past—forever young: cutting-edge biochemical and microbiological tools for cultural heritage conservation, *Appl Microbiol Biotechnol*, **102(16)** (2018) 6815-6825, http://doi.org/10.1007/s00253-018-9121-3.

4 Tanrıverdi M, *Conservation practices on manuscripts through the example of Konya Regional Library of manuscripts Work*, M S thesis, University of Pamukkale, Denizli, 2022.

5 Badalamenti N, Bruno M, Formisano C & Rigano D, Effect of germacrene-rich essential oil of *Parentucellia latifolia* (l.) caruel collected in central Sicily on the growth of microorganisms inhabiting historical textiles, *Nat Prod Commun*, **17(4)** (2022) 1934578X221096963.

6 Fouda A, Abdel-Maksoud G, Abdel-Rahman M A, Salem S S, Hassan S E D & El-Sadany M A H, Eco-friendly approach utilizing green synthesized nanoparticles for paper conservation against microbes involved in biodeterioration of archaeological manuscript, *Int Biodeterior Biodegradation*, **142** (2019) 160–169, https://doi.org/10.1016 /j.ibiod.2019.05.012

7 Fouda A, Abdel-Maksoud G, Saad H A, Gobouri A A, Mohammedsaleh Z M & Abdel-Haleem El-Sadany M, The efficacy of silver nitrate (AgNO3) as a coating agent to protect paper against high deteriorating microbes, *Catalysts*, **11(3)** (2021) 310, https://doi.org/10.3390/catal11030310.

8 Pasquarella C, Balocco C, Pasquariello G, Petrone G, Saccani E, Manotti P, Ugolotti M, Palla F, Maggi O & Albertini R, A multidisciplinary approach to the study of cultural heritage environments: Experience at the Palatina Library in Parma, *Sci Total Environ*, **536** (2015) 557–567, https://doi.org/10.1016/j.scitotenv.2015.07.105

9 Pavic A, Ilic-Tomic T, Pacevski A, Nedeljkovic T, Vasiljevic B & Moric I, Diversity and biodeteriorative potential of bacterial isolates from deteriorated modern combined-technique canvas painting, *Int Biodeterior Biodegradation*, **97** (2015) 40–50, https://doi.org/10.1016/j.ibiod.2014.11.012

10 American Society for Microbiology, Starch agar protocol, (2012), http://www.asmscience.org/content/ education/ protocol/protocol.3780. Accessed 29 March 2017

11 Sharma A K, Sharma V, Saxena J, Chandra R, Alam A & Prakash A, Isolation and screening of amylolytic bacteria from soil, *Int J Sci Res Agric Sci,* **2(7)** (2015a) 159–165, http://dx.doi.org/10.12983/ijsras-2015-p0159-0165

12 Sharma A K, Sharma V, Saxena J, Yadav B, Alam A & Prakash A, Isolation and screening of extracellular protease enzyme from bacterial and fungal isolates of soil, *Int J Sci Res Agric Sci,* **3(9)** (2015b) 334–340, http://doi.org/10.22207/JPAM.12.4.42

13 Atlas R M, *Handbook of Microbiological Media*. (Washington D.C., CRC Press), 2010.

14 Medina P & Baresi L, Rapid identification of gelatin and casein hydrolysis using TCA, *J Microbiol Methods,* **69(2)** (2007) 391–393, https://10.1016/j.mimet.2007.01.005

15 Imamoglu Ö, *Investigation of chitosanase activity and antifungal effect of bacillus strains isolated from different sources*, Dissertation, Universiy of Ege, İzmir, 2008.

16 Lane D J, 16S/23S rRNA sequencing. In: *Nucleic acid techniques in bacterial systematics*, (Stackebrandt E, Goodfellow M (Eds) John Wiley & Sons, Chichester) 1991, 115–175.

17 Noohi N & Papizadeh M, Study of biodeterioration potential of microorganisms isolated in the paintings storeroom of Mouze Makhsus museum, Golestan palace, Tehran, *Stud Conserv*, **68(7)** *(*2023) 720–730, https://doi.org/10.1080/ 00393630.2022.2118269.

18 Thompson J D, Gibson T J, Plewniak F, Jeanmougin F & Higgins D G, The CLUSTAL_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res*, **25(24)** (1997) 4876–4882, http://doi.org/10.1093/nar/25.24.4876.

19 Nicholas K B, *GeneDoc: Analysis and visualization of genetic variation*, Embnew news, 1997, **4(14)**.

20 Benson D A, Karsch-Mizrachi I, Lipman D J, Ostell J & Wheeler D L, GenBank, *Nucleic Acids Res*, **33(1)** (2005) 34–38.

21 Munn C, *Marine Microbiology: Ecology and Applications*, **3rd edn**, (CRC Press, New York) 2020.

22 Nguyen M T, Matsuo M, Niemann S, Herrmann M & Götz F, Lipoproteins in Gram-positive bacteria: abundance, function, fitness, *Front Microbiol*, **11** (2020) 582582, https://doi.org/10.3389/fmicb.2020.582582.

23 Logan N A & De Vos P, Bergey's Manual of Systematics of Archaea and Bacteria. In: *Bacillus*, (ed: Whitman WB, John Wiley & Sons NJ, USA) 2015, 1–164, https://doi.org/10.1002/9781118960608.gbm00530.

24 Stevens H, Brinkhoff T, Rink B, Vollmers J & Simon M, Diversity and abundance of Gram positive bacteria in a tidal flat ecosystem, *Environ Microbiol,* **9(7)** (2007) 1810–1822, http://doi.org/10.1111/j.1462-2920.2007.01302.x.

25 Krakova L, Chovanova K, Selim S A, Simonovicova, Puskarova A, Makova A & Pangallo D, A multiphasic approach for investigation of the microbial diversity and its biodegradative abilities in historical paper and parchment documents, *Int Biodeterior Biodegradation*, **70** (2012) 117–125, https://doi.org/10.1016/j.ibiod.2012.01.011.

26 Raeisnia N, Arefian E & Amoozega M A, Microbial Community of an 11th century manuscript by both culture-dependent and -independent approaches, *Microbiol*, **91(3)** (2022) 313–323, https://doi.org/10.1134/S00262617 22300117.

27 Alves P D D, de FariaSiqueira F, Facchin S, Horta C C R & Victoria J M N, Kalapothakis E, Survey of microbial enzymes in soil, water, and plant microenvironments, *Open Microbiol J*, **8(25)** (2014), https://doi.org/10.2174/ 1874285801408010025

28 Facchin S, Alves P D D, de Faria Siqueira F, Barroca T M, Victoria, J M N & Kalapothakis E, Biodiversity and secretion of enzymes with potential utility in wastewater treatment, *Open J Ecol*, **3(1)** (2013) 34–47, https://doi.org/10.4236/oje.2013.31005

29 Jeszeova L, Benzova R, Gulistikova M, Siskova A, Kisova Z, Plany M, Krakova L, Bauerova-Hlinkova V & Pangallo D, Biocleaning of historical documents: The use and characterization of bacterial enzymatic resources, *Int Biodeterior Biodegradation,* **140** (2019) 106–112, https://doi.org/10.1016/j.ibiod.2019.03.017.

30 Karakasidou K, Nikolouli K, Amoutzias G D, Pournou A, Manassis C, Tsiamis G & Mossialos D, Microbial diversity in biodeteriorated Greek historical documents dating back to the 19th and 20th century: A case study, Microbiology Open, **7** (2018) e596, https://doi.org/10.1002/mbo3.596.

31 Savoldelli S, Catto C, Villa F, Saracchi M, Troiano F, Cortesi P & Cappitelli F, Biological risk assessment in the History and Historical Documentation Library of the University of Milan, *Sci Total Environ*, **790** (2021) 148204, https://doi.org/10.1016/J.SCITOTENV.2021.148204.

32 Lopez-Miras M, Pinar G, Romero-Noguera J, Bolivar-Galiano F C, Ettenauer J, Sterflinger K & Martin-Sanchez I, Microbial communities adhering to the obverse and reverse sides of an oil painting on canvas: Identification and evaluation of their biodegradative potential, *Aerobiologia*, **29(2)** (2013) 301–314, https://doi.org/10.1007/s10453-012-9281-z

33 Di Bella M, Randazzo D, Di Carlo E, Barresi G & Palla F, Monitoring biological damage on paper-based documents in the historical archive of the Palermo astronomical observatory, *Conserv Sci Cult Herit*, **15(2)** (2015) 85–94, https://doi.org/10.6092/issn.1973-9494/7121.

34 Behdani S, Bahreini M, Mohammadi P, Shokrzadeh, Sharifmoghadam M M & Sabet Jazari A, Study of bacterial contamination of some exquisite written works of Astane Qudse Razavi Documentation Center, *Iran J Biol*, **34(2)** (2021) 182–192.

35 Gatson J W, Benz B F, Chandrasekaran C, Satomi M, Venkateswaran K & Hart M E, *Bacillus tequilensis* sp. nov., isolated from a 2000-year-old Mexican shaft-tomb, is closely related to *Bacillus subtilis*, *Int J Syst Evol Microbiol*, **56(7)** (2006) 1475–1484, https://doi.org/10.1099/ijs.0.63946-0.

36 Lech T, Evaluation of a parchment document, the 13th century incorporation charter for the city of Krakow, Poland, for microbial hazards, *Appl Environ Microbiol*, **82(9)** (2016) 2620–2631, https://doi.org/10.1128/AEM.03851-15.

37 Zajac I, Szul, J & Gutarowska B, The effect of ethylene oxide and silver nanoparticles on photographic models in the context of disinfection of photo albums, *J Cult Herit*, **51** (2021) 59–70, https://doi.org/10.1016/j.culher.2021.07.003.

38 Liu Y, Lai Q, Göker M, Meier-Kolthoff J P, Wang M, Sun Y, Wang L & Shao Z, Genomic insights into the taxonomic status of the *Bacillus cereus* group, *Sci Rep*, **5(1)** (2015) 1–11, https://doi.org/10.1038/srep14082.

39 Sugiyama J, Kiyuna T, Nishijima M, An K D, Nagatsuka Y, Tazato N, Handa Y, Hata-Tomita J, Sato Y, Kigawa R & Sano C, Polyphasic insights into the microbiomes of the Takamatsuzuka Tumulus and Kitora Tumulus, *J Gen Appl Microbiol*, **63(2)** (2017) 63–113, http://doi.org/10.2323/jgam.2017.01.007.

40 Pinar G, Krakova L, Pangallo D, Piombino-Mascali D, Maixner F, Zink A & Sterflinger K, Halophilic bacteria are colonizing the exhibition areas of the Capuchin Catacombs in Palermo Italy, *Extremophiles*, **18(4)** (2014) 677–691, https://doi.org/10.1007/s00792-014-0649-6.

41 Kim Y H, Choi K H, Hong J Y, Lee J M, Kim S J, Jo C W & Jeong S Y, Investigation of microorganisms deteriorating ancient ola leaf manuscripts, *Restaurator*, **41(3)** (2020) 119–129, https://doi.org/10.1515/res-2020-0004.

42 Vandamme P, Bernarde, J F, Segers P, Kersters K & Holmes B, New Perspectives in the Classification of the Flavobacteria: Description of *Chryseobacterium* gen. nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev., *Int J Syst Evol Microbiol*, **44(4)** (1994) 827–831, http://doi.org/10.1099/00207713-44-4-827.

43 Izaguirre-Anariba D E &Sivapalan V, *Chryseobacterium indologenes*, an emerging bacteria: A case report and review of literature, *Cureus*, **12(1)** (2020) e6720, https://doi.org/10.7759/cureus.6720.

44 Sırıken B & Öz V, *Pseudomonas aeruginosa*: özellikleri ve quorum sensing mekanizması, *J Food Feed Sci-Tech*, **18** (2017) 42–52.

45 Tao Y, Zhou Y, He X, Hu X & Li D, *Pseudomonas chengduensis* sp. nov., isolated from landfill leachate, *Int J Syst Evol Microbiol*, **64(1)** (2014) 95–100, http://doi.org/10.1099/ijs.0.050294-0.