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Research article

Uncovering the microbial diversity of Czech Republic archives: A study of metabolically active airborne microbes

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ABSTRACT

Despite the diligent efforts of libraries, archives, and similar institutions to preserve cultural monuments, biodeterioration continues to pose a significant threat to these objects. One of the main sources of microorganisms responsible for the biodeterioration process is the presence of airborne microorganisms. Therefore, this research aims to monitor and compare outcomes of both culture-dependent (utilising various cultivation strategies) and culture-independent approaches (RNA-based sequencing) to identifying metabolically active airborne microorganisms in archives in the Czech Republic. Through this study, several species that have the potential to pose risks to both cultural heritage objects and the health of institution employees were found. Additionally, the efficacy of different cultivation media was demonstrated to be varied across archive rooms, highlighting the necessity of employing multiple cultivation media for comprehensive analyses. Of noteworthy importance, the resuscitating-promoting factor (Rpf) proved to be a pivotal tool, increasing bacterial culturability by up to 30% when synergistically employed Reasoner's 2A agar (R2A) and R2A + Rpf media. Next, the study emphasises the importance of integrating both culture-dependent and culture-independent approaches. The overlap between genera identified by the culture-dependent approach and those identified also by the culture-independent approach varied from 33% to surpassing 94%, with the maximum alignment exceeding 94% in only one case. Our results highlight the importance of actively monitoring and assessing levels of microbial air contamination in archives to prevent further deterioration of cultural heritage objects and to promote improved conditions for employees in archives and similar institutions.

1. Introduction

Air quality, whether indoors or outdoors, plays a pivotal role in human health, as individuals are consistently exposed to a myriad of airborne microorganisms and pollutants [1,2]. While outdoor air quality has been a subject of extensive study and regulation, the importance of indoor air quality is increasingly recognized, given that individuals spend up to 90% of their time in indoor

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environments. It thus becomes essential to monitor the indoor air quality across various building types, such as schools, houses, and hospitals, and even within separate rooms of the same building [3].

Special consideration could be given to institutions such as libraries, archives, and museums that are dedicated to preserving countless cultural artefacts for centuries. These institutions create unique microenvironments, characterised by their specific functional purpose [1,4]. Despite concerted efforts, these artefacts remain vulnerable to microbial degradation caused by fungi and bacteria [5]. The contamination of the artefacts is largely influenced by the presence of microorganisms in the air, which in turn is primarily determined by the internal conditions of the institutions. These conditions, including the presence of air filtration systems, as well as the control of temperature and humidity levels, are critical in mitigating this issue [4,6,7].

Fungi, due to their mycotoxin production and allergenic potential, are significant biological agents contributing to air pollution, and play a key role in the deterioration of cultural heritage objects and in health complications among employees [8]. Allergic symptoms such as itchy eyes and throat and nasal congestion can occur when handling dusty cultural artefacts [9]. In the past, it was estimated that roughly a third of archive employees can develop fungal allergies, which is twice the rate observed in the general population [10]. Moreover, fungal spores settle on the surfaces of objects and can secrete extracellular enzymes, while their hyphae can cause mechanical damage to the materials [11]. Unlike bacteria, which typically require higher humidity to thrive, limiting their impact on the deterioration of cultural heritage objects compared to fungi. Nonetheless, bacteria are frequently found in archives and similar facilities, and can lead to the deterioration of materials and health disorders [6]. Thus, a thorough evaluation of air quality in cultural heritage preservation institutions should incorporate microbiological analyses, including both fungal and bacterial contamination.

Past research has investigated the microbial contamination in cultural heritage preservation institutions, with studies focusing on archives [6], museums [12,13], and libraries [1,7]. Notably, based on our knowledge, these studies predominantly employed culture-dependent approaches, which may not provide an exhaustive insight into the air contamination in these environments [14]. Despite this, several strategies exist to enhance the culturability of microorganisms. For instance, besides widely-utilised media such as nutrient agar (NA) and tryptic soy agar (TSA) [14], one could consider using nutrient-poor media, which may prove more appropriate for slow-growing species (e.g., Reasoner's 2A agar) [15–17]. Other tactics involve reducing nutrient concentrations, prolonging incubation time [18], or integrating growth-promoting factors such as resuscitating-promoting factor (Rpf) [19]. Rpf, a protein with a molecular weight ranging from 16 to 17 kDa, is primarily synthesised by GC-rich Gram-positive bacteria, particularly those belonging to the phylum Actinomycetota (former Actinobacteria) [19,20].

Over the last two decades, the field of cultural heritage has been progressively gravitating towards a culture-independent approach using molecular methods [21], with most studies focusing on DNA analysis. Although this provides information on living and dead cells, RNA analysis could provide more insightful results by detecting only metabolically active microorganisms [22,23]. However, such RNA-based studies remain limited in all environmental matrices, despite evidence of their potential in identifying active taxa and revealing differences in community structures compared to the overall community [24,25].

In light of this, our research aims to fill the research gap and provide a more comprehensive understanding of levels of air contamination by metabolically active bacteria and fungi in cultural heritage preservation institutions We adopted a combination of a culture-dependent approach, using diverse strategies to enhance cultivability, alongside a culture-independent (RNA-based) approach to find the answers to the following questions: i) To what degree will there be concurrence between the outcomes derived from culture-dependent and culture-independent approaches? ii) Will the experiment reveal significant disparities between different cultivation media? iii) Will it be possible to identify species in the air that have the potential to cause harm to both artefacts and employees within the preservation institution?

2. Materials and methods

2.1. Preparation of cultivation media

Four cultivation media were used for the microscopic fungi: malt extract agar (MEA), potato dextrose agar (PDA), Sabouraud agar (SDA), and yeast extract glucose chloramphenicol agar (YGC) (all Hi-media, Czech Republic). Four different cultivation media were also used for bacteria: Reasoner's 2A agar (R2A), R2A + Rpf (VWR International, USA), tryptic soy agar (TSA), and TSA + Rpf (Hi-media, Czech Republic). Except for YGC, antibiotics were added to all of the above media. Chloramphenicol (Duchefa Biochemie, Netherlands) was added to the fungal media and nystatin (Duchefa Biochemie, Netherlands) to the bacterial media. In addition, all the cultivation media were prepared with 10-fold diluted nutrients than the concentrations indicated in the manufacturer's technical documentation to promote the growth of slow-growing species, thereby enhancing overall microbial diversity [26].

2.1.1. Rpf preparation

The preparation of Rpf from *Micrococcus luteus* was inspired by the studies of Su et al. [27] and Lopez Marin et al. [19]. Briefly, the bacterial strain *M. luteus* NCTC 2665 was cultured in lactate minimal medium (LMM) containing (per litre): 10.0 g Lithium L-lactate, 4.0 g NH_4Cl, 1.4 g KH_2PO_4, 1.0 g Inosine, 0.04 g Thiamine B1, 0.03 g $MgSO_4$, 0.02 g L-Methionine, 0.005 g Biotin, and 1.0 mL of trace element solution. The trace element solution contained (per litre): 0.785 g $MnCl_2{\cdot}4H_2O$, 0.375 g $CuSO_4{\cdot}5H_2O$, 0.183 g $FeSO_4{\cdot}7H_2O$, 0.089 g $ZnSO_4{\cdot}7H_2O$, and 0.029 g $Na_2MoO_4{\cdot}2H_2O$. To begin the culture process, freshly grown *M. luteus* was pre-inoculated in 20 mL of LMM and inoculated on a shaker at 120 rpm at 28 °C for 3–4 days. Next, 1 mL of the prepared inoculum (OD = 1) was transferred into 200 mL of LMM and incubated again under the same conditions until the culture reached an absorbance of $A_{600} = 2.9$, representing the point of highest protein content in the medium [19]. The medium was then centrifuged at 5000×*g*, 10 min, 25 °C, and the supernatant

was filtered through 0.2 μm filter funnels (VWR International, USA). The filtrate was then further centrifuged using a 3 kDa Amicon Ultra® 15 mL centrifugal filter to concentrate the protein content, and the filter was washed with phosphate buffer. The concentrated Rpf was subsequently diluted with phosphate buffer to a volume three times less than that of the processed LMM medium. The obtained Rpf growth factor was stored in a freezer at −20 °C until further use.

2.2. Sampling

Sampling was scheduled in the morning to capitalise on the extended overnight period when the rooms were unoccupied, concurrently minimising the chance of human presence and movement during the sampling process. Air samples were collected from two rooms within each archive, specifically the State Regional Archive in Trebon and the Moravian Provincial Archive in Brno. These rooms house cultural heritage objects, including audio-visual materials. The characteristics of each individual room can be found in table S1. The collection of air samples involved using an MAS-100 Eco aeroscope (Merck Millipore, Germany) positioned at a height of approximately 1 m above the ground, where cultural artefacts were stored on shelves. For a culture-independent approach, 3000 L of air were gathered using pre-autoclaved PTFE filters (porosity 0.22 μm, diameter 90 mm, Merck Millipore, Germany). These filters were then aseptically divided into quarters in the laboratory, with two quarters designated for RNA isolation. This resulted in a total of six samples for sequencing per room per archive. In contrast, for a culture-dependent approach, 750 L of air were directly collected onto cultivation media. This was done since the RNA isolation was performed from quarters of the filters equivalent to 750 L of air. All sampling procedures were conducted in triplicate to ensure robustness and reliability of the results.

2.3. Culture-dependent approach

The bacterial cultivation media were incubated at 30 °C for 10–14 days, and the media designed for capturing microscopic fungi were incubated at 25 °C for the same duration. This incubation period was chosen based on the understanding that prolonged culture times enhance the likelihood of successfully cultivating slow-growing species [26]. Colony-forming unit (CFU) counts were performed after the incubation period. Due to the significantly higher colony numbers observed in Trebon compared to Brno, distinct colony identification procedures were utilised for each archive. In samples from the Brno archive, where a low quantity of microorganisms was detected (CFU/plate <30), individual CFUs were identified using MALDI-TOF MS or Sanger sequencing. Conversely, when encountering a high quantity of microorganisms (CFU/plate >30), as noted in the Trebon archive, the CFUs were collected together, and DNA isolation was performed on the mixed sample.

2.3.1. MALDI-TOF MS

The bacterial colonies obtained from the Brno archive were inoculated on fresh cultivation media, followed by identification using MALDI-TOF MS with an Autoflex Speed mass spectrometer (Bruker Daltonics, Bremen, Germany) and database Biotyper 3.1 (Bruker Daltonics, Bremen, Germany). A solution of alpha-cyano-4-hydroxycinnamic (HCCA, final concentration 10 mg mL^{-1}, Bruker Daltonics, Bremen, Germany) in organic solvent (500 μL acetonitrile, 250 μL nuclease-free water, and 250 μL 10% trifluoroacetic acid, all ThermoFisher, Waltham, MA, USA) was used as the matrix. The samples were spotted on an MTP 384 polished steel plate (Bruker Daltonics, Bremen, Germany) using extended direct transfer, i.e., spots with colonies were recoated with 2 μL of 70% formic acid within 30 min, and after drying, the spot was recoated with 2 μL of HCCA matrix. When there was a failed identification (i.e., no peaks were found or identification was at the "not reliable" level), DNA was isolated from each individual colony and prepared for Sanger sequencing. Since the numbers of fungi from Brno were low, they were all directly identified by Sanger sequencing.

2.3.2. Isolation of DNA from individual colonies for Sanger sequencing

The DNA isolation from individual colonies (from the Brno archive) was performed using a commercial InstaGene Matrix kit (Bio-rad Laboratories, Inc., USA) with several modifications. After adding the InstaGene Matrix and incubation at 56 °C for 30 min, glass beads of 425–600 μm (Sigma-Aldrich s.r.o., USA) were added, and homogenisation was performed at 6 m/s for 40 s. The extracted DNA was further used for the PCR, followed by sample preparation for Sanger sequencing according to the instructions from SEQme s.r.o.

2.3.3. Isolation of DNA from mixed culture for Illumina MiSeq sequencing

The colonies from Trebon were collected in 2 mL of saline solution from which DNA was isolated using a PureLink Genomic DNA Mini Kit (Invitrogen, USA). The microtubes containing the 2 mL mixture of colonies and saline solution were centrifuged at 12,000×*g* for 3 min. The supernatant was removed, and Glass beads of 425–600 μm (Sigma-Aldrich s.r.o., USA), lysozyme solution (5 mL of saline solution and 100 mg of lysozyme (Serva, Germany)), and Lysozyme digestion buffer were added to the pellet. Subsequently, homogenisation was performed at 6 m/s for 40 s. This was continued according to the instructions for the commercial Pure Link Genomic DNA Mini Kit with one modification in the last step, in which the DNA was eluted into 70 μL of nuclease-free water. The extracted DNA was further used for the PCR, followed by preparation for Illumina MiSeq sequencing.

2.3.4. DNA amplification and sequencing preparation

The DNA from individual colonies (Brno archive) and the mixed cultures (Trebon archive) was amplified using a polymerase chain reaction using specific primers for 16S rRNA (bacteria): forward primer 515FBAF 5-GTGYCAGCMGCNGCGG-3 and reverse primer 926R 5-CCGYCAATTYMTTTRAGTTT-3 [28], and for the ITS2 region (fungi): forward primer 5.8S_Fun 5-AACTTTYRRCAAYGGATCWCT-3 and reverse primer ITS4_Fun 5-AGCCTCCGCTTATTGATATGCTTAART-3 (all Sigma-Aldrich, St. Louis, MO, USA) [29].

For both Sanger and Illumina MiSeq sequencing, the first PCR mix contained nuclease-free water, 1 μM of each of the appropriate primers, 0.02 U/μL of KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), and 2 μL of template in a total reaction volume of 15 μL, and each sample was run in 8 replicates. The thermocycling program was as follows: 5 min of denaturation at 95 °C, followed by 30 cycles of 20 s at 98 °C, 15 s at 56/50 °C for the 16S rRNA/ITS2 region, and 15 s at 72 °C; the final extension was run at 72 °C for 5 min. Afterwards, the replicates were merged and concentrated using the commercial kit Genomic DNA Clean & Concentrator (ZYMO Research, Irvine, CA, USA). The results were checked using 1.5% (*w/v*) agarose gel electrophoresis (120 V, 60 min).

Next, for Sanger sequencing, the concentration of DNA was measured using a nanodrop, and the samples were prepared according to the instructions from SEQme s.r.o., where the Sanger sequencing was performed. For Illumina MiSeq sequencing, index PCR was performed. The PCR mix contained nuclease-free water, 1 μM of each of the appropriate primers, 0.02 U/μL of KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), and 1 μL of template in a total reaction volume of 25 μL. The thermocycling program was as follows: 5 min of denaturation at 95 °C, followed by 13 cycles of 20 s at 98 °C, 15 s at 50 °C, and 15 s at 72 °C; the final extension was run at 72 °C for 5 min. The results of PCR were checked using 1.5% (*w/v*) agarose gel electrophoresis (120 V, 60 min). The prepared amplicons were sent to the University of Fairbanks, Alaska for the Illumina MiSeq sequencing.

2.4. Culture-independent approach

2.4.1. Isolation of total RNA

As was mentioned above, the PTFE filters were quartered. From two quarters, RNA was isolated using an RNeasy PowerWater kit (Qiagen, Germany), with a modification in the last step, in which the RNA was eluted into 70 μL of nuclease-free water. The extracted RNA was further used for transcription into cDNA, followed by preparation for Illumina MiSeq sequencing.

2.4.2. RNA transcription, amplification, and sequencing preparation

The RNA was transcribed into cDNA using an EliZyme OneS Kit (Elizabeth Pharmacon, Czech Republic), which allows reverse transcription and PCR in one tube. The first PCR mix contained nuclease-free water, 400 nM of each of the appropriate primers (same as for DNA amplification), 25 μL of EliZyme OneS kit Mix, 2.5 μL of RTase, and 5 μL of template in a total reaction volume of 50 μL, and each sample was run in 3 replicates. The thermocycling program was as follows: 10 min of reverse transcription at 50 °C, 2 min of denaturation at 95 °C, followed by 40 cycles of 10 s at 95 °C, 10 s at 56/50 °C for 16S rRNA/ITS2 region, and 15 s at 72 °C; the final extension was run at 72 °C for 5 min. Afterwards, the replicates were merged and prepared for Illumina MiSeq sequencing in the same manner as DNA samples from mixed cultures (chapter 2.4.3.).

2.5. Data processing

2.5.1. Sanger sequencing

The received sequences were aligned using the software Molecular Evolutionary Genetics Analysis (MEGA) [30]. Subsequently, the appropriate microorganisms were identified using the Basic Local Alignment Search Tool algorithm (National Center for Biotechnology Information, NCBI).

2.5.2. Illumina MiSeq sequencing

The taxonomy was assigned to the sequences using the programming language R [31]; the package DADA2 [32]. The procedure was adopted from DADA2 pipeline version 1.16. for bacteria and from DADA2 pipeline version 1.8. for fungi. The sequences of forward and reverse primers were removed, and the 16S rRNA sequences were filtered according to the following parameters: truncLen = (233, 170), maxN = 0, maxEE = (2,2), truncQ = 2, while the ITS region sequences were filtered according to the following parameters: maxN = 0, maxEE = c(2,7), truncQ = 2, minLen = 50. Next, chimeras were identified and removed by the "consensus" method. The bacterial taxonomy was assigned to amplicon sequence variants (ASVs) using the silva_nr_v132_train_set.fa.gz database [33]. The fungal taxonomy was assigned using the UNITE database [34]. To eliminate potential errors, sequences that differed by only one base (16S rRNA gene)/two bases (ITS region) were concatenated and represented as the more abundant of the two.

2.6. Multivariate statistical analysis

Prior to conducting statistical analysis, negative controls were subtracted in the R programming language, and the data obtained from Illumina MiSeq were normalized using the "compositional" method. The statistical analysis was carried out within the same programming language using the packages vegan [35] and phyloseq [36]. Shannon diversity indices were plotted to assess alpha biodiversity for the culture-dependent and culture-independent approaches used to identify microorganisms and for the different cultivation media in the Trebon archive, and differences were examined using the Tukey HSD test. Additionally, a permutation multivariate analysis of variance, known as PERMANOVA, based on the Bray-Curtis distance, was performed to investigate differences in microbial community structures between the two approaches and the two sampled rooms in the archives. Furthermore, a pairwise PERMANOVA was conducted to explore differences in microbial communities between the various cultivation media used in the Trebon archive. For the Brno archive, where the culture-dependent approach resulted in a lower number of colonies, a Wilcoxon rank-sum test was employed to identify differences between the cultivation media and the archive's rooms. Microbial community structures in each room, using the culture-independent approach, were assessed using the PERMANOVA method, as with the Trebon

archive. Stacked bar charts were created for both archives, illustrating the relative abundances of fungal and bacterial genera in the air. Moreover, heatmaps were constructed, displaying the 30 most abundant fungal and bacterial species in the archive's air. However, the heatmaps excluded the culture-dependent approach in Brno, due to the low number of species obtained. Lastly, Venn diagrams were created to show the intersections of genera identified using different cultivation media, as well as the intersections of genera identified through both culture-dependent and culture-independent approaches.

3. Results

3.1. Microbial diversity in Trebon archive

3.1.1. Bacteria

The total numbers of bacteria captured on the cultivation media in each room of the archives are shown in table S2. The findings reveal that higher levels of bacterial contamination were detected in the lower room. To provide visual context, fig. S1 shows Petri dishes containing TSA/10 medium (10x diluted with added nystatin).

At the genus level, the intersections of bacterial genera captured by different cultivation media are depicted in Fig. 1 for the lower room (a) and the upper room (b). The findings from the figure show that the R2A/10 medium exhibited the highest efficacy at capturing genera in the lower room, accounting for 73% of all identified genera. Remarkably, together with the R2A/10+Rpf medium, this percentage increased to 91%. In contrast, TSA/10 and TSA/10+Rpf only managed to capture 48% of all genera. In the upper room, both the R2A/10 and R2A/10+Rpf media proved to be the most effective, each capturing 63% of all genera, and even 93% when used in combination. Conversely, TSA/10, together with TSA/10+Rpf cultivation media, captured only 48% of all genera.

Next, the diversities obtained by different cultivation media were examined using the Shannon diversity index (fig. S2). Subsequently, a pairwise PERMANOVA was performed to test whether the type of cultivation medium had an effect on the structure of the bacterial communities. The results revealed a statistically significant difference at the 95% level between all pairs of cultivation media except for R2A/10 and R2A/10+Rpf ($p_{adj} = 0.055$).

Moreover, Shannon diversity index analysis was employed to compare bacterial diversities obtained by the culture-independent approach (RNA analysis) and the culture-dependent approach. As anticipated, the culture-independent approach (RNA analysis) gave higher diversity than the culture-dependent approach (fig. S3). To further compare the two approaches and rooms, a PERMANOVA analysis was conducted, which confirmed a statistically significant difference ($p_{adj} = 0.001$) in the structures of the bacterial communities obtained by the two approaches, and also between the archive rooms themselves.

In parallel, graphical representations were employed to visualize the relative abundances of the identified bacterial genera for both approaches. The relative abundance of bacterial genera captured on various cultivation media in both archive rooms is depicted in Fig. 2a. In the upper room, the genus *Bacillus* predominates on R2A/10+Rpf and TSA/10 media, whereas the genus *Staphylococcus* predominates on TSA/10+Rpf media, and the genus *Paracoccus* predominates on R2A/10 media. Moving to the lower room, the genera *Stenotrophomonas* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* are the dominant ones on TSA/10 and TSA/10+Rpf media, with an additional high abundance of the genus *Pantoea* on TSA/10 media. Furthermore, the latter two genera, along with *Curtobacterium*, also exhibit high abundance on R2A/10 and R2A/10+Rpf media. In contrast, Fig. 2b exhibits a relative abundance of bacterial genera obtained by culture-independent techniques (RNA analysis) from the same archive and its rooms. In this case, the most abundant genera include *Lactobacillus*, *Paracoccus* and *Streptococcus*.

Additionally, a heatmap was created to show the 30 most abundant bacterial species in the Trebon archive (fig. S4). The heatmap reveals that 4 of these most abundant species were only identified by the culture-independent approach (*Neisseria* spp., *Streptococcus* spp., *Scytonema*_UTX_2349 spp. and "Not identified" species identified only at the family level – Burkholderiaceae), while 2 species were only captured by the culture-dependent approach (*Lelliottia* spp. and *Kocuria* spp.).

To examine the overall overlap of bacterial genera captured in the Trebon archive between the two approaches, we find that over 94% of the genera identified by the culture-dependent approach were also identified by the culture-independent approach (fig. S5). The genera that have been identified only through the culture-dependent approach include, in addition to the above-mentioned genera, *Kocuria* and *Lelliottia*, the genera *Verticia* and *Agrococcus*.

3.1.2. Fungi

The total numbers of fungi captured on the cultivation media in each room of the archives are shown in table S3. Notably, the

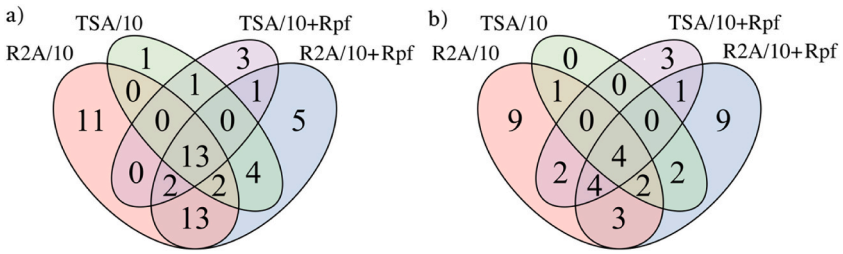

Fig. 1. Intersection of bacterial genera identified in Trebon archive using different cultivation media in a) lower room, b) upper room.

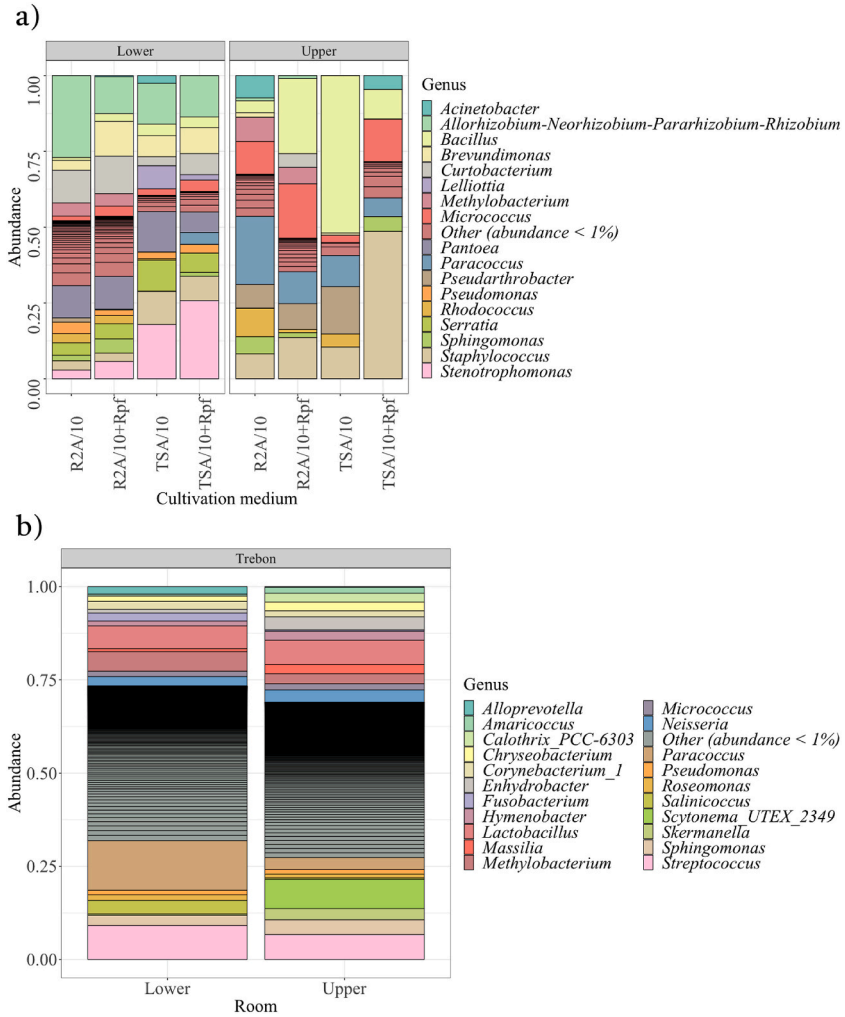

Fig. 2. Relative abundance of bacterial genera isolated from two different rooms (lower, upper) in the State Regional Archive in Trebon using a) culture-dependent approach, b) culture-independent approach.

findings show higher levels of fungal contamination in the upper room. For a visual representation, Petri dishes containing MEA/10 medium (10x diluted with added chloramphenicol) are shown in fig. S6.

The intersections of fungal genera captured with various cultivation media are visualized in Fig. 3, representing the lower room (a) and the upper room (b) at the genus level. Notably, the MEA/10 medium demonstrated its efficacy at capturing the highest number of genera in the lower room, accounting for 60% of all identified genera. Conversely, in the upper room, the YGC/10 medium exhibited

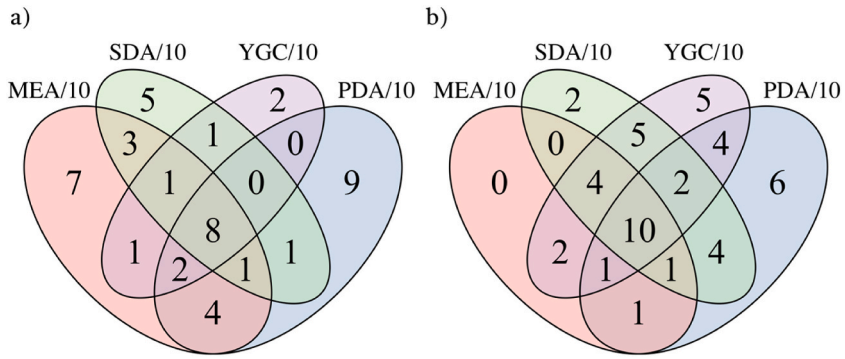

Fig. 3. Intersection of fungal genera identified in Trebon archive using different cultivation media in a) lower room, b) upper room.

superior capturing capabilities, identifying the highest number of genera, approximately 70% of all identified genera.

In order to investigate the influence of different cultivation media on the diversity of fungi, the Shannon diversity index was employed (fig. S7). Subsequently, a pairwise PERMANOVA analysis was conducted to examine the impact of these media on the structure of fungal communities, The results indicated that there was no statistically significant difference at the 95% level between any pair of cultivation media.

In line with bacteria, the examination of fungal diversity using the Shannon diversity index revealed that the culture-independent method (RNA analysis) gave higher diversity than the culture-dependent method (fig. S8). Since the data followed a normal distribution, this outcome was further validated by Tukey's HSD, which confirmed a statistically significant difference ($p_{adj} < 0.05$) between the two approaches. Furthermore, the same statistical analysis showed no statistically significant difference ($p_{adj} > 0.05$) in diversity between the two archive rooms. Differences in fungal community structures were observed using PERMANOVA, where a statistically significant difference was found in the use of the approach ($p = 0.001$), even between the rooms analysed ($p = 0.001$).

A relative abundance of fungal genera in different rooms of the Trebon archive, captured using a culture-dependent approach with various cultivation media, is depicted in Fig. 4a. The predominant genera in the lower room were *Bulleromyces*, *Cladosporium* and *Sporobolomyces*, while in the upper room, the genera *Alternaria*, *Bulleromyces* and *Cladosporium* were the most abundant. The relative abundance of fungal genera in different rooms of the Trebon archive, identified using a culture-independent approach, is displayed in Fig. 4b. In the lower room, the most abundant genera were *Malassezia*, *Mycosphaerella*, *Sporobolomyces*, and *Vuilleminia*, while in the upper room, the most abundant genera were *Mycosphaerella* and *Phaeosphaeria*.

Furthermore, a heatmap displaying the 30 most abundant fungal species identified in the Trebon archive was generated (fig. S9). Notably, the culture-independent approach captured 8 of the most abundant species, including *Rhodosporidiobolus* spp., *Malassezia globosa*, *Bensingtonia ciliata*, *Vuilleminia comedens*, *Ceratobasidium* spp., *Talaromyces piceae*, *Phaeosphaeria* spp., and *Mycosphaerella*

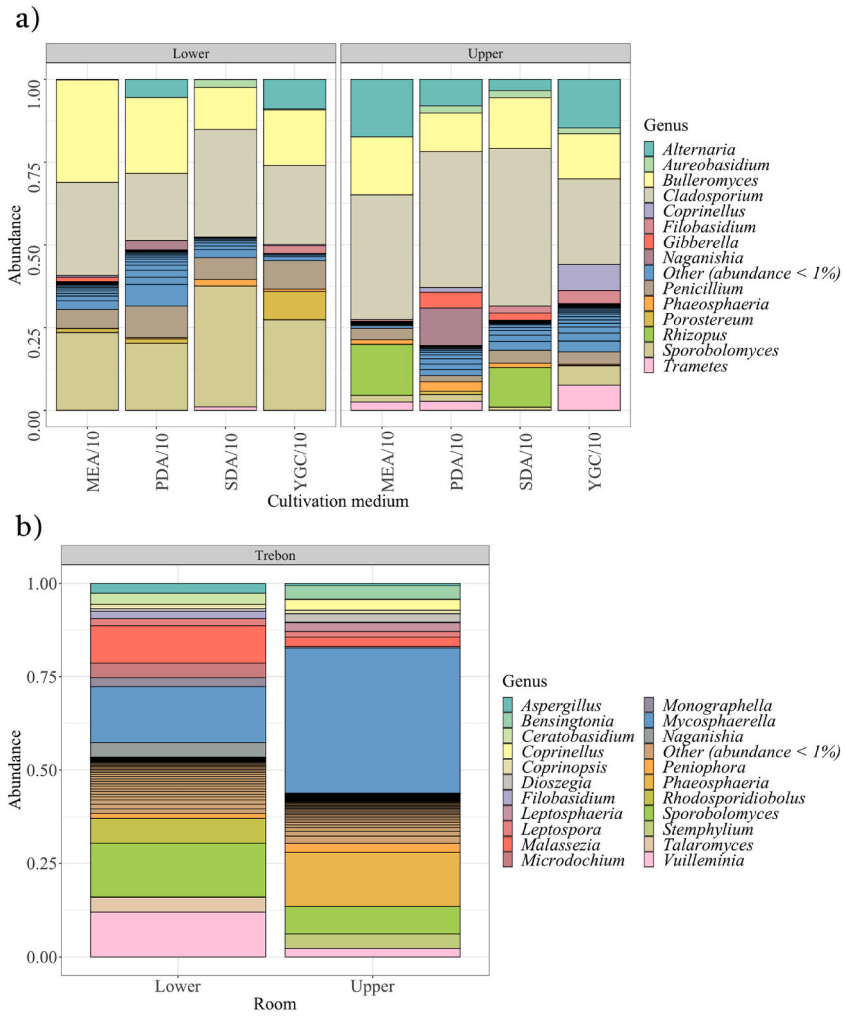

Fig. 4. A relative abundance of fungal genera isolated from two different rooms (lower, upper) in the State Regional Archive in Trebon using a) culture-dependent approach, b) culture-independent approach.

tassiana, which were not detected by the culture-dependent approach. Conversely, the culture-dependent approach captured 7 species that were not detected by the culture-independent approach, including *Sporisorium* spp., *Bulleromyces albus*, *Coprinellus xanthothrix*, *Giberella tricincta*, *Fusarium langsethiae*, *Penicillium bialowiezense*, *Alternaria alternata*, and *Cladosporium delicatulum*.

The overlap of all fungal genera identified by culture-dependent and culture-independent approaches is presented in fig. S10. The Venn diagram shows that 50% of the genera identified through the culture-dependent approach were also captured by the culture-independent approach.

3.2. Microbial diversity in Brno archive

3.2.1. Bacteria

The total numbers of bacteria captured on the cultivation media in each room of the Moravian Provincial Archive in Brno are shown in table S4. Regrettably, a certain proportion of bacterial colonies captured in the Brno archive failed to regrow on the fresh cultivation media, resulting in a loss of approximately 33% of the colonies. It is important to note that the table shows the original counts (without the loss). To provide visual context, fig. S1 shows Petri dishes containing TSA/10 medium (10x diluted with added nystatin).

The Venn diagram in Fig. 5a (lower room) and 5b (upper room) illustrates the overlapping bacterial genera identified using various cultivation media. In the lower room, the R2A/10 medium proved to be the most effective, capturing the majority of genera, accounting for 53% of the total. This percentage increased to 84% when used together with the R2A/10+Rpf. In contrast, the TSA/10+Rpf medium exhibited the highest efficiency in the upper room, achieving an efficiency of 63%, and even 88% when used in combination with the R2A/10 or TSA/10 medium.

As the CFU counts obtained in the Brno archive using a culture-dependent approach were lower than that in the Trebon archive, the Wilcoxon rank-sum test was conducted to analyse the differences between cultivation media. The statistical analysis indicated no statistically significant differences ($p > 0.05$) between any pair of cultivation media. Similarly, there was no statistically significant difference ($p > 0.05$) between the rooms under analysis. With the culture-independent approach, the PERMANOVA analysis did not confirm the statistically significant differences ($p = 0.145$) in the structure of fungal communities obtained in the two rooms.

Similar to the analysis conducted for the Trebon archive, the relative abundance of bacterial genera was evaluated for the Brno archive. The relative abundance of bacterial genera captured by the culture-dependent approach using various cultivation media is shown in Fig. 6a. In the lower room, both TSA/10 and TSA/10+Rpf media detected *Micrococcus* as the most abundant genus, while R2A/10 was dominated by genus *Kocuria* and R2A/10+Rpf by genera *Cellulomonas* and *Chryseobacterium*. In the upper room, *Micrococcus* was the dominant genus on R2A/10 and R2A/10+Rpf media, while TSA/10 and TSA/10+Rpf media were dominated by the genera *Macrococcus* and *Agrococcus*, respectively. A relative abundance of bacterial genera obtained through sequencing analysis is shown in Fig. 6b, with *Delftia* and *Sphingobium* being the most abundant genera in both, the lower and upper rooms.

Next, a heatmap was created of the 30 most abundant bacterial species identified by the RNA analysis in the Brno archive (fig. S11). The heat map shows that *Delftia* spp. and *Sphingobium yanoikuyae* were the most abundant species in both rooms of the archive. It also shows that all 30 of the most abundant species were found in both rooms.

The overlap between bacterial genera identified in the Brno archive by both culture-dependent and culture-independent approaches is shown in fig. S12. Of the genera identified by the culture-dependent approach, 55% were also identified by the culture-independent approach. These genera included *Bacillus*, *Brachybacterium*, *Chryseobacterium*, *Kocuria*, *Luteimonas*, *Marmoricola*, *Microvirga*, *Microbacterium*, *Rhodococcus*, *Sphingomonas*, *Staphylococcus*, and *Variovorax*.

3.2.2. Fungi

The total numbers of fungi captured on the cultivation media in each room of the Moravian Provincial Archive in Brno are shown in table S5. For a visual representation, Petri dishes containing MEA/10 medium (10x diluted with added chloramphenicol) are shown in fig. S6.

In this case, the intersection of fungal genera captured using different cultivation media was limited to the lower room (Fig. 7), since only one colony was captured in the upper room. Notably, the lower room exhibited remarkable efficiency with the YGC/10 medium, capturing a significant proportion of microscopic fungi, amounting to 75% of the total.

Statistical analysis using the Wilcoxon rank-sum test was also used to compare the efficacy of the different cultivation media used for isolating fungi from the Brno archive. Similar to the bacterial analysis, the result did not show any statistically significant

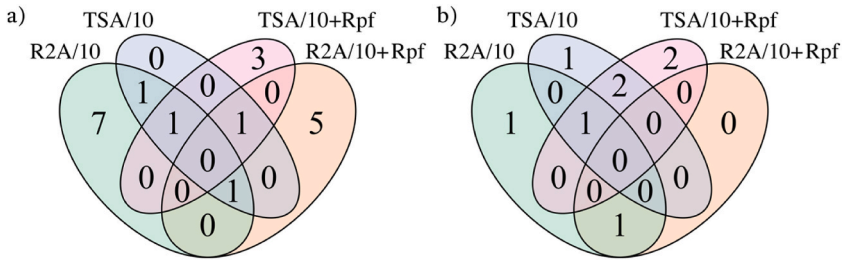

Fig. 5. Intersection of bacterial genera identified in Brno archive using different cultivation media in a) lower room, b) upper room.

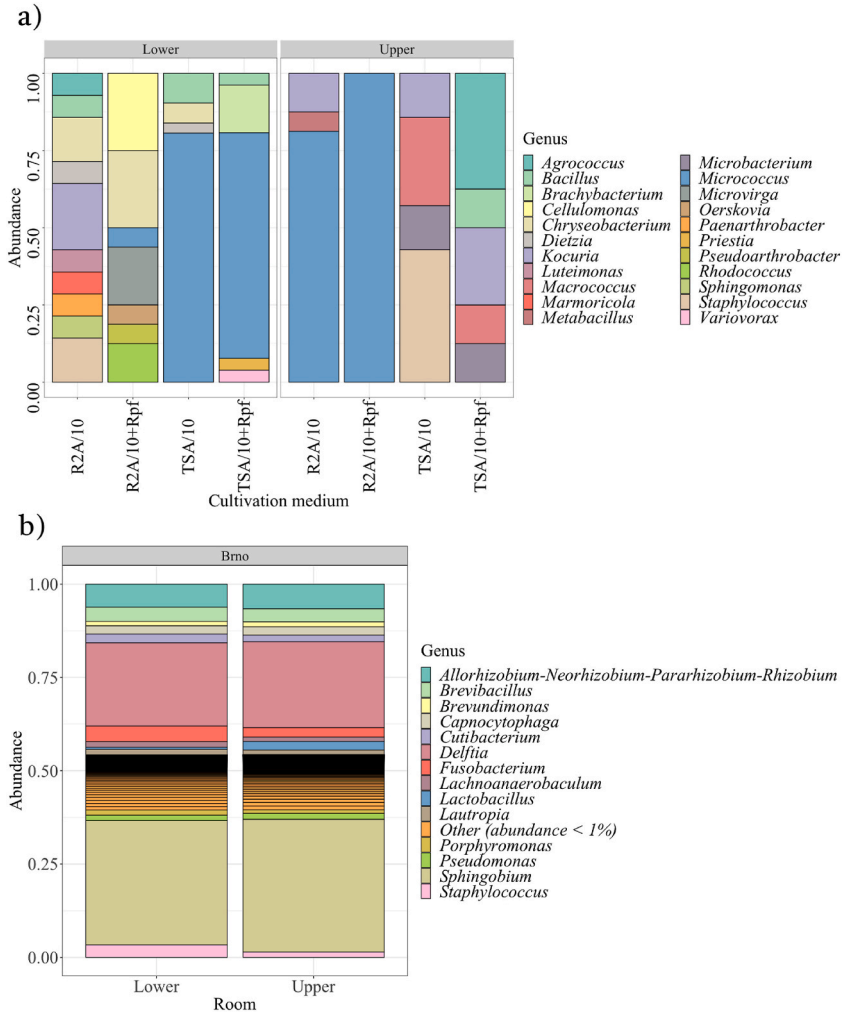

Fig. 6. Relative abundance of bacteria genera isolated from two different rooms (lower, upper) in the Moravian Provincial Archive in Brno using a) culture-dependent approach, b) culture-independent approach.

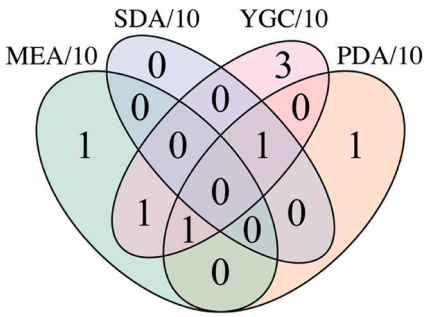

Fig. 7. Intersection of fungal genera identified in Brno archive using different cultivation media in lower room.

differences ($p > 0.05$) between any pair of cultivation media. Statistical analysis could not be conducted to assess the differences between rooms using the culture-dependent approach, as only one colony was captured in the upper room. Meanwhile, no statistically significant difference ($p = 0.185$) in fungal community structure between the archive rooms was confirmed using the culture-

independent approach.

A relative abundance of fungal genera in different rooms of the Brno archive, captured by a culture-dependent approach using various media, is shown in Fig. 8a. In the lower room, the dominant genera included *Cladosporium*, *Cryptococcus* and *Penicillium*, while only one genus, *Pseudopithomyces*, was captured in the upper room. In addition, the relative abundance of fungal genera identified using a culture-independent approach is shown in Fig. 8b. The genera *Aspergillus*, *Malassezia* and *Toxicocladosporium* were the most abundant in the lower room, while *Malassezia* and *Neoascochyta* were the most abundant genera in the upper room.

A heatmap was also generated to visualize the 30 most abundant fungal species identified through RNA analysis (fig. S13). The heatmap demonstrates notable variations between the different rooms. In the lower room alone, 13 species were identified among the top 30 most abundant species. These species include *Aspergillus halophilicus*, *Athelia* spp., *Filobasidium oeirense*, *Hannaella luteola*, *Heterobasidion orientale*, *Knufia endospora*, *Oxyporus corticola*, *Rhodotorula mucilaginosa*, *Strobilurus esculentus*, and *Toxicodocladosporium irritans*, as well as three "Not identified" species, two identified at the order level (Entylomatales and Malasseziales), and one identified at the family level (Trichomeriaceae). On the other hand, 7 species were found only in the upper room: *Cladophialophora humicola*, *Debaryomyces hansenii*, *Erythrobasidium hasegawianum*, *Knufia perforans*, *Leptospora* spp., and *Neoscochyta graminicola*, and *Penicillium chrysogenum*.

The fungal genera identified in the Brno archive by both culture-dependent and culture-independent approaches were compared, and the overlap is shown in fig. S14. It was found that one-third (approximately 33%) of the genera identified by the culture-dependent approach were also identified by the culture-independent approach. These genera included *Cryptococcus*, *Penicillium*, and

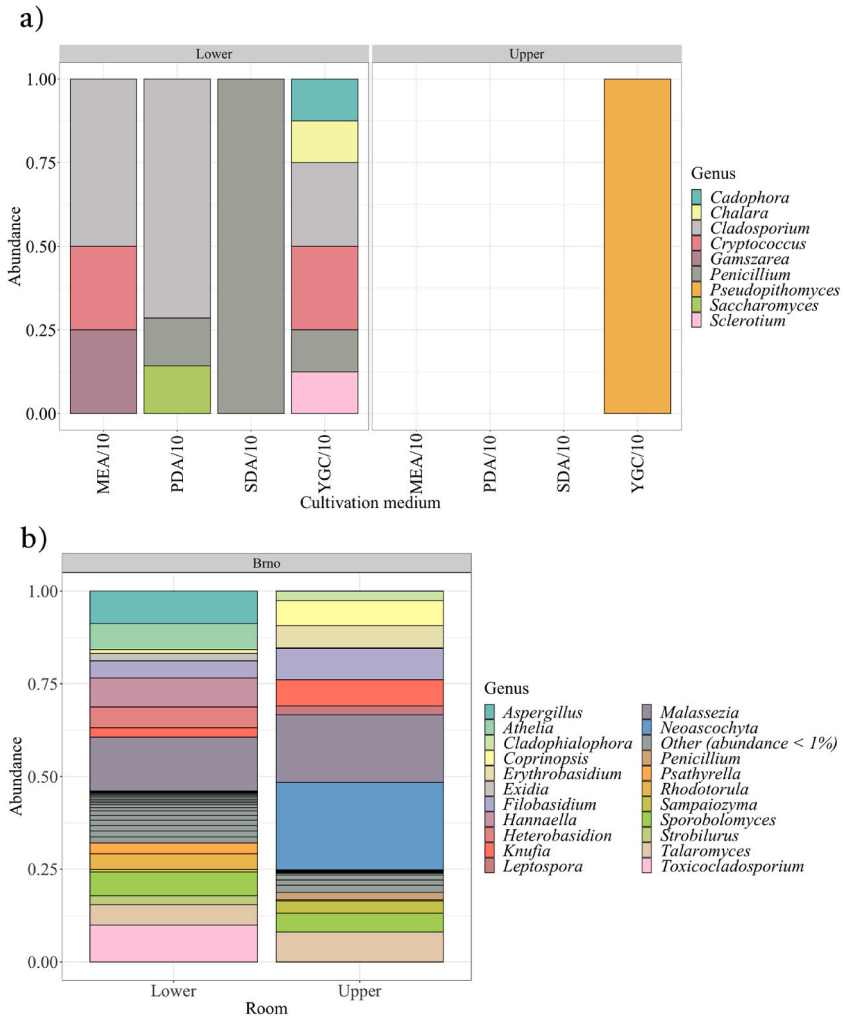

Fig. 8. Relative abundance of fungal genera isolated from two different rooms (lower, upper) in the Moravian Provincial Archive in Brno using a) culture-dependent approach, b) culture-independent approach.

Pseudopithomyces.

4. Discussion

In this study, the microbial contamination of the air in two archives located in the Czech Republic was investigated employing two approaches, a culture-dependent and culture-independent one. Notably, the number of studies focusing on the identification of microorganisms in the air near cultural heritage objects remains limited, despite the frequent attribution of air as the primary source of microbial contamination of such objects [7,37]. Moreover, most of the existing studies have only utilised a culture-dependent approach with only a few studies have used multiple cultivation media in their sampling process, overlooking the potential for diverse outcomes, even within identical environments, that can arise from utilising different cultivation media [13,38]. Enhancing the obtained number of CFUs and the diversity of species through a culture-dependent approach can improve the likelihood of discovering previously uncultivable species and offer more profound insights into the community dynamics within a given environment [13,26].

An example of using a combination of multiple media would be the study by Dziurzynski et al. [13] monitored museum air using the same aeroscope (MAS-100 Eco, Merck) as we employed. However, in this particular study, seven different cultivation media for capturing bacteria were utilised: Blickfeldt agar, Brain Heart Infusion, Frazer medium, Lysogeny broth, Nutrient agar, R2A medium, and Yeast extract agar. Notably, this study underscores the significance of employing various media in the sampling process by highlighting the discernible differences among individual types. The highest capturability was documented with R2A medium (similar to our study in the Trebon archive and the lower room of the Brno archive), with the predominant taxa identified as the genera *Bacillus*, *Micrococcus,* and *Pseudomonas*. These genera were also found to be abundant in our research, with *Bacillus* and *Micrococcus* being captured using a culture-dependent approach in both archives (Figs. 2a and 6a).

The application of diverse cultivation media, as exemplified by the aforementioned study by Dziurzynski et al. [13], represents a recommended strategy for augmenting the efficacy of microbial species capture through cultivation techniques within environmental contexts. Additional strategies encompass nutrient dilution, prolonged cultivation time, or the addition of growth factors into the media [16,18]. In our study, we considered these recommendations and implemented a 10-fold nutrient dilution of varied cultivation media, tested the addition of the resuscitation-promoting factor Rpf, and extended cultivation times up to 14 days for both bacteria and fungi. All these conditions have also been previously tested and verified in the master thesis by Lisa [39].

Of the bacterial media used, the TSA medium proved to be one of the most commonly used in cultural heritage research (used, for instance, in studies by Puskarova et al. [40], Pietrzak et al. [41], Krakova et al. [42], and many others). However, our study also introduced the use of the R2A medium, which has been relatively rare in previous studies (based on the review by Branysova et al. [14], which discusses the biodeterioration of various cultural heritage objects). The advantages of this medium for monitoring bacteria in the environment are discussed, for example, in the paper by Ciric et al. [43], where the authors mention that this low-nutrient medium is most widely used for water and environmental monitoring, because its low nutrient concentration supports a wide range of bacteria, and especially the growth of slow-growing species. This suggests that the R2A medium is also better suited for monitoring the environment around cultural heritage objects. In accordance with our findings, the R2A/10 medium emerged as the most efficacious of the bacterial cultivation media assessed in most examined rooms (Figs. 1 and 5). However, the TSA/10 medium supplemented by Rpf was more effective in the upper room in the Brno archive, highlighting the importance of using multiple cultivation media (Fig. 5b).

The addition of Rpf into the cultivation media yielded an enhancement in our ability to capture a broader spectrum of bacterial genera. This enhancement is also exemplified in the study led by Lopez Marin et al. [19], in which the authors successfully captured 51 novel bacterial species from soil using an R2A medium supplemented with Rpf, as well as media derived from the soil's water-soluble fraction with Rpf. These newly captured species were primarily classified within the phyla Actinomycetota, Pseudomonadota, and Bacteroidota. Importantly, in our own investigation, it is worth highlighting that all the genera exclusively captured on Rpf-supplemented media also belong to these specific phyla, including Bacillota. Our results thus emphasise the importance of utilising more diverse cultivation media, along with supplementary additives, to improve overall efficacy (increase capturability). This statement is also supported by the results for microscopic fungi, since even in each room of the Trebon archive, a different medium showed the highest efficiency, the MEA/10 medium in the lower room and the YGC/10 medium in the upper room (Fig. 3b). The assertion gains additional support from the observation that, aside from the MEA/10 media employed in the Trebon's archive's lower room (Fig. 3a) and the SDA/10 media utilised in Brno's lower room (Fig. 7), numerous genera were exclusively captured by specific media – those being the only media to capture them.

When considering indoor environmental quality, in the Czech Republic, regulations specifically addressing indoor environmental quality in archives are currently lacking. However, it's important to note that Decree No. June 2003 Coll., a legal document issued by the Czech government, outlines requirements for the indoor environment of buildings. It explicitly excludes areas with elevated cleanliness demands, such as hospitals or manufacturing plants, covered by a different decree. According to Decree No. June 2003 Coll., applicable to our study, the concentration of bacteria in the air should not exceed 500 CFU/m^3, and similarly, the concentration of fungi should not exceed 500 CFU/m^3. As far as our results are concerned, this condition was met in both rooms of both archives, and even for all cultivation media. Remarkably, the Brno archive exhibited microbial counts several orders of magnitude lower than those in the Trebon archive (tables S2–S5). This difference in contamination levels could potentially be attributed to variations in the air conditioning systems between the two archives. While the Trebon archive lacks an air conditioning system, the Brno archive implements an air purification mechanism involving a chemisorbant material and ISO Coarse filters ≥70 % (G4). Additional factors influencing airborne microorganism concentrations encompass temperature and relative humidity [44,45]. The fluctuations and variance in these environmental conditions play a pivotal role in shaping the dynamics of microorganism diffusion and growth [46]. It

is these fluctuations that may contribute to the considerably higher CFU counts observed in the Trebon archive. Specifically, on the day of sampling, the temperature within the Trebon archive was approximately 8 °C above the annual average temperature. Conversely, in the Brno archive, deviations from annual climatic values were marginal, potentially explaining the divergence in CFU counts between the two archives.

Besides quantifying the levels of microbial contamination in the archives, it is crucial to also identify the microorganisms and their potential impact on both employee health and the preservation of cultural heritage [47,48]. In the following text, we focused on abundant species identified by both culture-dependent and culture-independent methods in the Trebon archive, specifically those ranking among the 30 most abundant species which were identified to the species level. Of the bacterial species it concerns *Enhydrobacter aerosaccus*, *Paracoccus yeei*, and *Skermanella aerolata* (fig. S4). For fungi, the success of identification at the species level surpassed that of bacteria. Consequently, we refined our focus exclusively to those fungal species that ranked among the 30 most abundant species through both approaches (fig. S9) and whose genus exhibited a relative abundance exceeding 1% (Fig. 4). Notably, the fungal species *Filobasidium magnum*, *Naganishia diffluens*, *Phaeosphaeria triglochinicola*, and *Sporobolomyces roseus* were well-represented.

The bacterium *Enhydrobacter aerosaccus* is a common member of the human skin microbiota [49]. Within the genus *Enhydrobacter*, which comprises only two species [50], a notable predisposition exists for the biodeterioration of audio-visual materials [23]. In our previous study Branysova et al. [51], the species *E. aerosaccus* was identified on audio-visual materials. *Paracoccus yeei* stands as the sole opportunistic pathogenic species within the bacterial genus *Paracoccus.* Despite lacking a direct association with specific diseases, instances of its isolation from patients suffering from peritonitis, myocarditis post-heart transplant, corneal transplant, bacteraemia, keratitis, otitis, or dermatological lesions have been recorded [52]. Furthermore, the genus *Paracoccus* has been detected on diverse cultural heritage objects, including wall paintings, wooden artefacts [53], textiles [41], library air and historical books. This presence often manifests as discernible pink and beige stains on paper [54]. Next, *Skermanella aerolata,* a bacterial species isolated from the air [55], gained clinical significance in 2018 when the first documented case of human infection was reported. Remarkably, this infection, identified as necrotizing fasciitis, was likely contracted through contact with soil rather than airborne exposure [56]. In the context of cultural heritage objects, *S. aerolata* has also been successfully isolated from marble cultural heritage objects [57].

Turning our attention to the fungal genera identified in the Trebon archive, the genus *Phaeosphaeria* appear to pose minimal threats to both employees and cultural heritage objects, as indicated by the available literature. In contrast, the genera *Filobasidium* and *Naganishia*, which were formerly part of the genus *Cryptococcus* [58], certainly warrant closer attention. Species of the genus *Naganishia* are ubiquitous saprophytic yeasts that can act as opportunistic pathogens [59], causing a range of diseases from superficial to deep-seated infections. Noteworthy, our study identified the representative *N. diffluens*, associated with atopic dermatitis [60]. Considering cultural heritage objects, this species has been detected in cave air containing mural paintings [61]. Conversely, the widely distributed species *Filobasidium magnum* exhibits colonisation potential in the nasal cavity of paediatric cancer patients, and instances of otomycosis have been reported [62]. In the realm of cultural artefacts, *F. magnum* has been isolated from canvas paintings undergoing deterioration [63].

The genus *Sporobolomyces* encompasses an opportunistic pathogenic species known for inducing profound skin infections [64]. Its representative *S. roseus*, identified in this study, is classified among the opportunistic pathogens, posing a potential threat to immunosuppressed patients [65]. Beyond clinical contexts, this genus has also been identified in our previous study, both on audio-visual materials and in the air of four archives in the Czech Republic [29]. Furthermore, the genus *Sporobolomyces* is associated with the formation of red spots due to its carotenoid production, affecting stone monuments [66].

Shifting our focus to the Brno archive, where only a few species were obtained through the culture-dependent approach, we compare those captured through cultivation at all with those with an abundance greater than 1% identified through a culture-independent approach (Figs. 6b and 8b). These were the bacterial genus *Staphylococcus* and the fungal genus *Penicillium,* for which the species *Penicillium chrysogenum* was identified at the species level. While discussing the genus *Staphylococcus*, it is noteworthy to acknowledge that the identification was limited to the genus level, potentially leading to misconceptions regarding health risks for workers. This limitation arises from the inclusion of both commensal and pathogenic species within this genus [67]. Regarding cultural heritage, as previously discussed in our prior investigation [51], it is crucial to underscore the deleterious impact of this genus. It is known that the genus not only possesses proteolytic enzymes capable of damaging audio-visual materials but is also linked to the degradation of various cultural artefacts, including textiles, wall paintings, canvases, wood, paper, and parchments [23].

The fungal genus *Penicillium*, a globally distributed and common fungal genus, primarily serves the natural function of organic matter decomposition [68]. Human pathogens within the genus *Penicillium* are very rare, with only two cases reported for *Penicillium digitatum* [69]. However, another species, *Penicillium chrysogenum*, identified in this study, has associations with sick building syndrome, manifesting symptoms such as allergic rhinitis, respiratory distress, and chest tightness in individuals residing in specific buildings [70,71]. In addition, this species exhibits potent degradative properties [72] and has also been identified on different cultural heritage objects, including filmstrips [73].

The abundance of the aforementioned genera in the archive's air suggests potential hazards to both individuals and various cultural heritage objects. However, it is essential to note the risk of contracting the above-mentioned diseases is primarily relevant to the employees of archives, museums, libraries, and similar establishments, who are constantly exposed to this air, rather than occasional visitors [10]. Moreover, the health risks posed by these microorganisms are particularly concerning for individuals with weakened immunity.

In addition to the evaluation of the quantity of microorganisms in the air, and their taxonomic classification, the intersections between genera identified using a culture-independent approach (RNA analysis) and a culture-dependent approach were explored. For both bacteria and fungi, the intersections were not as high as expected (33–55%). It was only for the bacteria in the Trebon archive that

the percentage of genera identified by the culture-dependent approach that were also identified by the culture-independent approach reached 94%. The choice of primers and the omission of usage of multiple primer pairs might be the contributing factors for both domains. This is particularly relevant in environmental samples, where microbial identification pertains to an entire consortium rather than individual colonies [74,75]. For bacteria, a previous study by Varliero et al. [76] demonstrated that primers designed for the region V4–V5 region of 16S rRNA can underestimate the Actinomycetota phylum, which includes some of the genera exclusively identified by the culture-dependent approach. If these genera were not included in the intersection analysis, the percentage in the Brno archive would rise from 54.5% to 80%. Nevertheless, a comprehensive investigation comparing various regions of the 16S rRNA gene, including the V4–V5 region, found that this region along with the V4 region, exhibit the highest coverage within the bacterial domain [77].

Similarly, for fungi, a recommendation emerges to prioritise the ITS2 region due to its lower variability and the availability of more universal primers, resulting in reduced taxonomic bias. Notably, in our study, we employed the primers 5.8S_Fun and ITS4_Fun for generating ITS2 amplicons, with reported amplification efficiencies of 95% and 90%, respectively, across all fungal groups [78]. However, the use of the ITS4 primer has its pitfalls, as pointed out by Bellemain et al. [74] in their study, as the use of primer ITS4 resulted in a preferential amplification of the Ascomycota phylum, leading to an underestimation of other phyla, such as Basidiomycota. While in the Brno archive, there is only one Basidiomycota among the genera identified only by the culture-dependent approach, in the Trebon archive, Basidiomycota accounted for approximately half. Nevertheless, utilising multiple primer pairs can be cost-prohibitive when employing next-generation sequencing methods, as it significantly multiplies the number of samples to be sequenced. As demonstrated by our study, an effective approach may involve incorporating both culture-dependent and culture-independent methods in research to gain a more comprehensive understanding of microbial diversity.

In outlining future directions for research, we highlight avenues that hold great promise in advancing air quality monitoring within archives and other repositories of cultural heritage objects. A comprehensive understanding of the microorganisms present in these environments is crucial, and future investigations can significantly contribute to achieving this goal. For culture-dependent strategies, a key focus should be on optimising cultivation media to enhance the culturability of microorganisms, particularly for fungi — a facet often overshadowed by bacterial-centric approaches [79]. Introducing more efficient yet accurate next generation sequencing methods that will lead to cost savings, the combination of primers covering more variable regions or sequencing the whole marker genes, as discussed earlier, emerges as a noteworthy avenue for enhancing the precision of microbial assessments. These prospective research endeavours not only deepen our insights into microbial dynamics in such environments but also pave the way for developing targeted and accurate microbial analysis strategies.

5. Conclusion

Our study provides valuable insights into the microbial communities inhabiting the air of archives in the Czech Republic and emphasises the importance of considering cultivation media and microbial identification approaches when studying this specific niche. Through our investigation, numerous genera of fungi and bacteria were identified, and the necessity of employing both a culture-dependent and culture-independent approach was demonstrated. The culture-dependent approach underscored the importance of using a diverse range of cultivation media to capture a broader spectrum of microorganisms. However, our findings support the utilisation of RNA analysis, a culture-independent method, as it revealed a greater microbial diversity and highlighted the advantages of such approaches in microbial community studies. On the other hand, by combining both culture-dependent and culture-independent methods, we were able to present a more comprehensive understanding of microbial air contamination in cultural heritage preservation institutions, with a focus on archives. This integrated approach enabled us to identify genera that would have been missed by either method alone. Therefore, we encourage researchers to adopt a multi-faceted approach like ours when studying similar environments, as it significantly contributes to a more holistic and accurate assessment of microbial communities.

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Data availability statement

Sequences obtained from Illumina MiSeq sequencing were uploaded to the NCBI database under BioProject PRJNA1005643. Sanger sequences obtained from the individual colonies were deposited in Genebank, for bacteria submission SUB13762689, sequences OR462733 to OR462782, and for fungi submission SUB13762691, sequences OR460000 to OR460021.

CRediT authorship contribution statement

Tereza Branysova: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nicole Petru:** Investigation. **Marco A. Lopez Marin:** Methodology. **Milada Solcova:** Methodology. **Katerina Demnerova:** Resources. **Hana Stiborova:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27930.

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