

Article

# Assessment of Bacterial Contamination of Air at the Museum of King John III's Palace at Wilanow (Warsaw, Poland): Selection of an Optimal Growth Medium for Analyzing Airborne Bacteria Diversity

Mikolaj Dziurzynski <sup>1</sup>, Karol Ciuchcinski <sup>1</sup>, Magdalena Dyda <sup>2,3</sup>, Anna Szych <sup>1</sup>, Paulina Drabik <sup>2</sup>, Agnieszka Laudy <sup>4</sup> and Lukasz Dziewit <sup>1,\*</sup>

<sup>1</sup> Department of Environmental Microbiology and Biotechnology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland; mikolaj.dziurzynski@biol.uw.edu.pl (M.D.); k.ciuchcinski@student.uw.edu.pl (K.C.); aszych@biol.uw.edu.pl (A.S.)

<sup>2</sup> Research and Development for Life Sciences Ltd., Miecznikowa 1/5a, 02-096 Warsaw, Poland; magdalena.dyda@rdls.pl (M.D.); paulinawdrabik@gmail.com (P.D.)

<sup>3</sup> Department of Geomicrobiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

<sup>4</sup> Museum of King John III's Palace at Wilanow, Laboratory of Environmental Analysis, Stanislawina Kostki Potockiego 10/16, 02-958 Warsaw, Poland; alaudy@muzeum-wilanow.pl

\* Correspondence: ldziewit@biol.uw.edu.pl; Tel.: +48-22-554-14-06

Received: 3 September 2020; Accepted: 7 October 2020; Published: 13 October 2020



**Abstract:** There is no standardized protocol for the assessment of microbial air contamination in museums and other cultural heritage sites. Therefore, most museums conduct such assessments based on their own guidelines or good practices. Usually, microbial air contamination is assessed using only classical microbiology methods with the application of a single growth medium. Therefore, this medium should be carefully selected to limit any selective cultivation bias. Metabarcoding, i.e., a next-generation sequencing (NGS)-based method, combined with classical microbiological culturing was used to assess the effectiveness of various media applications in microbiological screening at the Museum of King John III's Palace at Wilanow (Warsaw, Poland). The obtained results indicated that when using a classical microbiology approach to assess the microbial air contamination at the museum, the selection of a proper growth medium was critical. It was shown that the use of rich media (commonly applied by museum conservators) introduced significant bias by severely underreporting putative human pathogens and the bacterial species involved in biodeterioration. Therefore, we recommend the use of other media, such as Frazier or Reasoner's 2A (R2A) medium, as they could yield more diverse communities and recovered the highest number of genera containing human pathogens, which may be suitable for public health assessments.

**Keywords:** air quality; cultural heritage; museum; next-generation sequencing; PCR amplicon

## 1. Introduction

Microbial indoor air contamination is attracting increasing attention from researchers. In 1955, in one of the first articles on this topic, Wells proposed that clean air should contain no more than  $1.5 \times 10^3$  CFU/m<sup>3</sup> in summer and  $4.5 \times 10^3$  CFU/m<sup>3</sup> in winter of microorganisms (bacteria and fungi) [1]. Other researchers proposed similar thresholds for clean, non-contaminated indoor air [2]. The common usage of such standards clearly indicates the importance of classical microbiology methods for indoor

air quality assessments. Unfortunately, this approach is highly variable and strongly dependent on the institution performing the analysis and the applied methodology. The very first step of such an analysis may involve various approaches, i.e., active (oftentimes involving the usage of various air samplers and impactors) [3] and passive sampling [4,5]. Furthermore, various media and incubation conditions may be applied. Significantly different results may, therefore, be produced, even for the same indoor area.

Novel metagenomic approaches of microbial biodiversity assessment, based on next-generation sequencing (NGS), constitute an alternative to the classical microbiology analyses. NGS-based methods have many advantages over classical ones. First and foremost, they allow for the detection of uncultivable microorganisms. Furthermore, these high-throughput screening methods allow for the detection of minorities among microbial species. Despite their strengths, NGS-based methods bear some flaws, such as their relatively high cost, the need for expertise in bioinformatic data analysis, and a lack of standard operating procedures (SOPs) for air quality assessments [6,7]. Currently, the most widely adopted NGS-based technique is metabarcoding, which uses the sequencing of PCR-amplified marker genes (e.g., 16S rRNA for bacteria and ITS1 or ITS2 for fungi) for the assessment of microbial biodiversity [8].

Museums are specific open public spaces where cultural heritage monuments are presented to a broad audience. At the same time, exhibitions are strictly protected to prevent their destruction. Biodeterioration and degradation are critical concerns in the conservation and restoration of historical art pieces at museums all over the world [9–12]. To prevent and counteract the effects of these processes, it is of utmost importance to identify the microbial species inhabiting historical art pieces and discover their biological activities that are influencing abiotic surfaces. Classical microbiology methods have been used for many years to achieve this goal [13]. While beneficial for many reasons, including their low cost and the possibility for the further biochemical analysis of isolated microorganisms, these methods also bear some significant limitations, as it is estimated that less than 1% of all microorganisms are cultivable [14–16].

It is also worth mentioning that microbial air contamination in museums may be hazardous to visitors. Museums usually constitute enclosed, air-conditioned spaces that are visited by a multitude of people each day. This may lead to the accumulation of potentially pathogenic microorganisms, which could be easily transmitted between visitors via the circulating air [17–19]. Therefore, reliable monitoring systems for assessing the microbiological contamination of air at museums are needed.

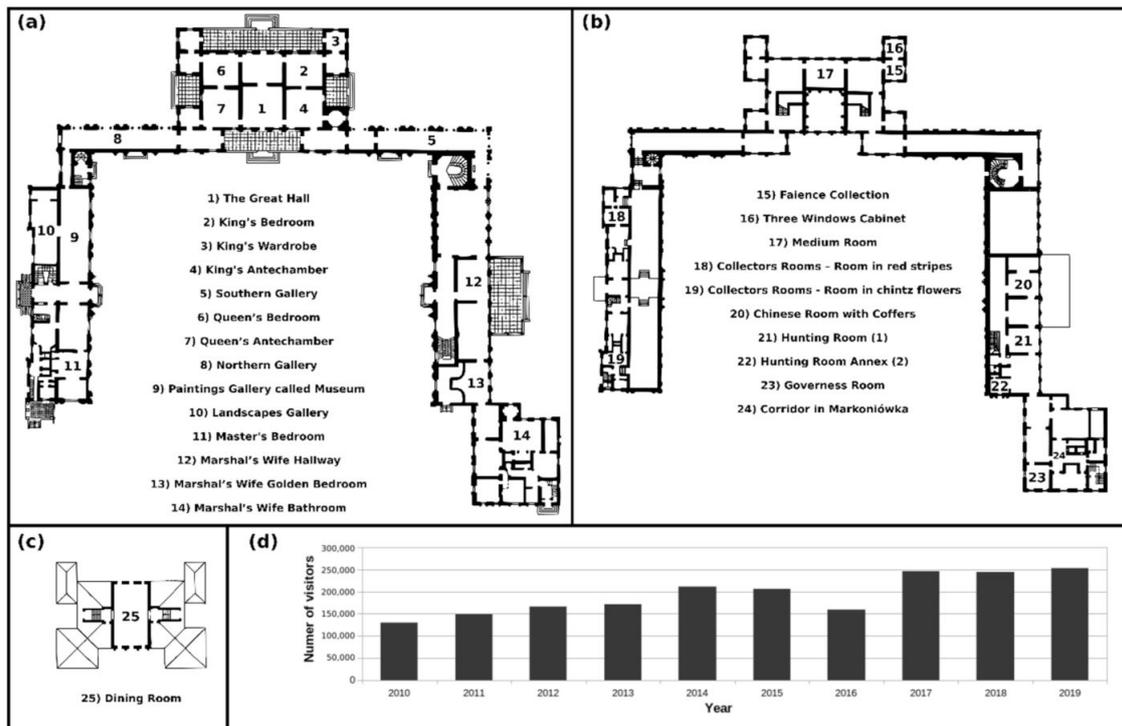
The Wilanow Palace in Warsaw (Poland), which was analyzed in this study, was initially bought in 1677 for King Jan III Sobieski and was transformed into an art gallery in 1805. Nowadays, this unique property is the oldest art gallery in Poland that is open to the public and is visited by hundreds of thousands of visitors each year. The Palace itself hosts more than 9100 different artworks, including paintings, sculptures, decorative arts, prints, and textile arts from the XVII–XIX centuries, with most of them being of high importance for European and world heritage. Since the early XXI century, the facades and interiors of the palace, as well as the park and forefield, have undergone extensive revitalization works. Additionally, the Wilanow Palace is a member of the Association of the Royal Residences of Europe (ARRE), which enables the cooperation and exchange of knowledge and experience about the preservation and development of cultural heritage.

In this study, we used metabarcoding to select the best culture media combinations for the detection of the highest number of bacterial genera. Therefore, the goal of this study was to show how metagenomic methods can improve classic microbiology approaches that are commonly employed for microbial screening in cultural heritage institutions.

## 2. Materials and Methods

### 2.1. Sample Collection

Sampling was conducted over the autumn and winter seasons of 2017–2018. In total, six sampling campaigns were conducted, with 25 exhibition rooms sampled each time. Sampling took place in rooms housing various types of cultural heritage objects. This approach was applied to minimize the material-dependent bias and to maximize the observed biodiversity. The exact locations of the sampling rooms are shown in Figure 1.



**Figure 1.** The plan of the Wilanow Palace rooms and the number of visitors in the last 10 years. Sections (a–c) show the locations of the sampling sites, i.e., rooms located on all three floors of the Wilanow Palace; (d) graph showing the number of visitors per year, starting from the year 2010.

Air samples were collected using the MAS-100 Eco device (Merck, Darmstadt, Germany) on agar plates with seven different bacterial growth media: Blickfeldt agar (BA), Brain Heart Infusion agar (BHI), Frazier agar (FA), lysogeny broth agar (LB), nutrient agar (NA), Reasoner's 2A agar (R2A), and yeast extract agar (YEA). Agar media were manufactured by BTL company (Lodz, Poland). At each sampling site, the device was positioned at 1.5 m above the ground using a tripod, and the airflow was set to 100 L per minute ( $\pm 2.5\%$ ). Each plate was inoculated with 50 L of air. In total, 1050 agar plates (150 plates per medium) were inoculated and were later subjected to further analyses.

### 2.2. Bacterial Cultivation Experiments

The agar plates were incubated at 37 °C for 48 h, following the Museum's regular practice for the detection of bacterial pathogens. The bacterial colonies on each plate were counted and the number of colony-forming units (CFU) per cubic meter of air was calculated with the Feller's correction taken into account.

### 2.3. Metagenomic DNA Isolation and Sequencing

After incubation, each plate was washed with 2 mL of saline (0.85% NaCl solution in water). The obtained suspensions were merged by growth media, which gave seven mixed suspensions in total, one for each growth medium. The suspensions were centrifuged (12,000× g, 5 min), the supernatant was discarded, and bacterial pellets were subjected to further analyses. DNA was isolated from 100 mg of bacterial pellets for each of the seven pools separately using the FastDNA<sup>®</sup> SPIN Kit for Feces and the FastPrep<sup>®</sup> Instrument (MP Biomedicals, Santa Ana, CA, USA). Region V3/V4 of the 16S rDNA was PCR-amplified with primers: L16SV3V4 (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'), and R16SV3V4 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'). The amplification was carried out in a Mastercycler Nexus GX2 thermocycler (Eppendorf, Hamburg, Germany) using KAPA HiFi DNA polymerase (KAPA Biosystems, Wilmington, MA, USA) with 5 ng of extracted DNA. For the DNA extracted from the bacteria grown on each type of medium, PCRs were conducted in triplicate and then mixed. The PCRs were prepared and performed as described previously [20]. The PCR products were quality-checked using electrophoresis in a 1.5% agarose gel. Seven PCR amplicons were then sequenced in a paired-end mode using a v3 chemistry kit with the Illumina MiSeq instrument (Illumina, San Diego, CA, USA) by the DNA Sequencing and Oligonucleotide Synthesis Laboratory—oligo.pl (Institute of Biochemistry and Biophysics, Polish Academy of Sciences).

### 2.4. Bioinformatics

The reads obtained from the MiSeq sequencer were first processed using fastp software in order to remove adaptor sequences [21]. Next, the reads were processed using the Qiime2 software package v. 2019.7 (<http://qiime2.org>) to perform quality-based trimming (`-p-trunc-len-f 285 -p-trunc-len-r 240`) and remove chimeras (`-p-min-fold-parent-over-abundance 3`) using the DADA2 library [22,23]. The remaining trimmed reads were treated as amplicon sequence variants (ASVs) [24]. Basic biodiversity indexes, such as Shannon's, Pielou's evenness, and Faith's indexes, were calculated for each of the samples using Qiime2 diversity (<https://github.com/qiime2/q2-diversity>), feature table (<https://github.com/qiime2/q2-feature-table>), and phylogeny (<https://github.com/qiime2/q2-phylogeny>) libraries. Taxonomies were assigned using a naive Bayes classifier from the feature-classifier (<https://github.com/qiime2/q2-feature-classifier>) library with the Silva database [25].

Other bioinformatic analyses were performed using custom Python scripts with matplotlib and seaborn libraries [26,27]. Statistical analysis was performed using R in the RStudio environment [28,29]. In order to determine significant differences between the colony-forming units (CFU) measured using methods of classical microbiology, the Kruskal–Wallis rank-sum test and the Mann–Whitney *U* test were used.

### 2.5. Systematic Literature Review for the Detection of Genera Hosting Human Pathogens

A systematic review of the available scientific literature was conducted in order to identify the bacterial genera hosting at least one species recognized as a human pathogen. The systematic review was conducted using the PubMed search engine according to the search scheme "(X) AND (pathogen) AND (human)," where X was a genus name. Additionally, each genus was checked for past name changes and reclassification. If such cases were found, previous genus names were also screened using the methodology described above. A genus was classified as "a genus containing pathogenic species" when at least one case study reported such a phenomenon.

### 2.6. Data Availability

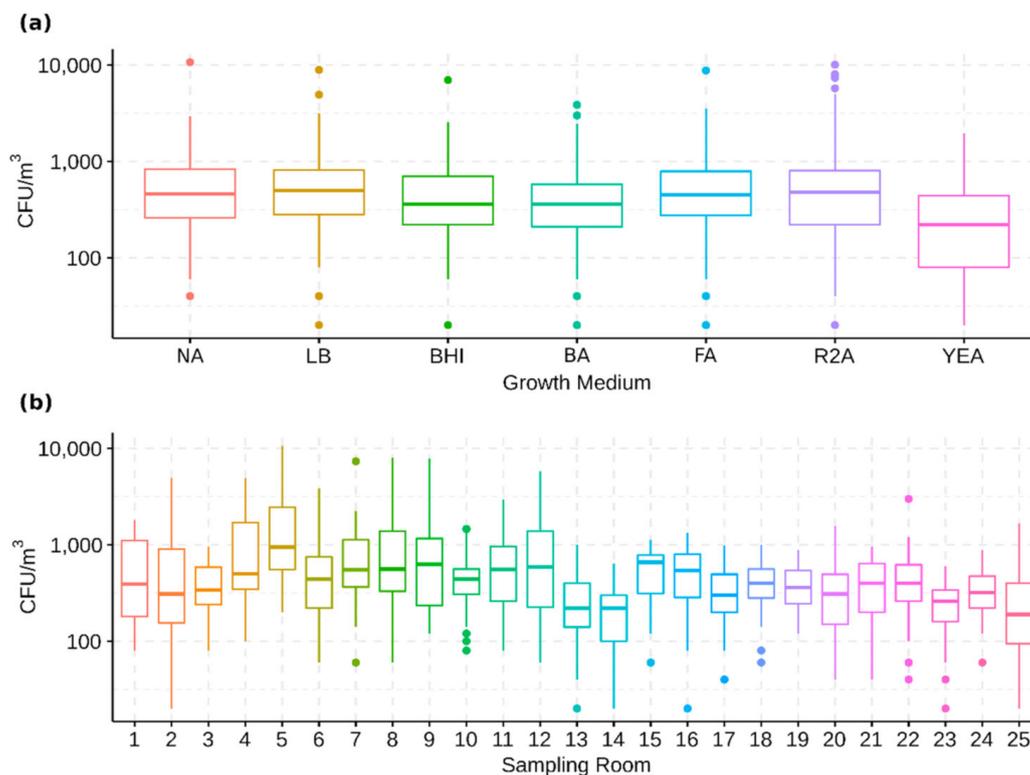
The next-generation sequencing data was deposited at the European Nucleotide Archive under study number PRJEB38331 and link [www.ebi.ac.uk/ena/data/view/PRJEB38331](http://www.ebi.ac.uk/ena/data/view/PRJEB38331).

### 3. Results and Discussion

#### 3.1. Assessment of Bacterial Contamination of Air—Quantitative Analysis Using the Classical Microbiology Approach

There are no special protocols for assessing bacterial air contamination in places of cultural heritage significance. Moreover, the European standard for microbiological air contamination in cleanrooms, which are facilities that are required to maintain extremely low levels of particulates (ISO 14698-1:2003), does not recommend any specific culture media for microbiological assessments. The only requirement established in these norms is that a non-selective medium is used. In this study, seven different growth media (LB, NA, BHI, YEA, BA, FA, and R2A) were tested for their suitability for use in the screening of microbiological air contamination. We focused on airborne mesophilic bacteria, as this group provides the best estimate for contamination with human pathogens [30].

Figure 2 presents the overall air contamination that was measured using classical microbiology methods. Pairwise statistical analysis between yields from different media highlighted the YEA medium as having the lowest global recovery rate. The pairwise Wilcoxon rank-sum tests with YEA were always statistically significant, with  $p$ -values  $< 10^{-6}$  (Table S1). This highlighted the fact that the YEA medium enabled the formation of significantly fewer colonies (mean CFU/m<sup>3</sup> equaled  $3.0 \times 10^2$ ) compared with the other investigated growth media (mean CFU/m<sup>3</sup> ranged between  $5.2 \times 10^2$  and  $9.3 \times 10^2$ ).



**Figure 2.** Box plots showing the measured CFU/m<sup>3</sup> distributions with a logarithmic scale. (a) Graph presenting the measured CFU/m<sup>3</sup> counts for each investigated medium. (b) Graph showing the CFU/m<sup>3</sup> counts by sampled rooms. The bold, central horizontal bars are the medians. The lower and upper limits of the box are the first and third quartiles, respectively. Points above and below the whiskers' upper and lower bounds are outliers. NA—nutrient agar, LB—lysogeny broth, BHI—brain heart infusion agar, BA—Blickfeldt agar, FA—Frazier agar, R2A—Reasoner's 2A agar, YEA—yeast extract agar.

The R2A, FA, and LB media showed the highest global recovery rates, with average CFU/m<sup>3</sup> values equal to  $9.3 \times 10^2$ ,  $7.1 \times 10^2$ , and  $7.0 \times 10^2$ , respectively. It is worth mentioning that the highest average CFU/m<sup>3</sup> was obtained for the R2A medium, which was designed to promote the growth of slow-growing heterotrophic bacteria. This observation is in line with the findings of other studies, highlighting the potential for the application of R2A medium in air contamination assessment protocols. In this case, the R2A medium could replace commonly used rich media, such as NA or tryptone soya agar (TSA) [31–33]. The benefit of using the R2A medium relies on its specific features, as a wide range of cultivable bacteria can be obtained due to the promoted growth of slow-growing species. In contrast, the diversity of cultivable isolates observed on rich media was lower, as the promoted growth of fast-growing bacteria suppressed the appearance of slow-growing ones.

The results obtained for R2A, FA, and LB media were in line with other works describing indoor environments. Brągoszewska et al. investigated indoor air pollution in human dwellings in Upper Silesia, Poland. According to their case study, the average microbiological air pollution in human dwellings was  $9.6 \times 10^2$  CFU/m<sup>3</sup> [34]. A more comprehensive analysis of microbiological air pollution in office buildings conducted in another study led by Brągoszewska reported recovery rates of  $1.4 \times 10^3$  CFU/m<sup>3</sup> in naturally ventilated offices and  $9.7 \times 10^2$  CFU/m<sup>3</sup> in offices with air conditioning [35]. A study by Kalwasińska et al. investigated microbiological air contamination in different rooms of a university library. By sampling different rooms with varying visitor accessibilities, the authors concluded that bacterial contamination is highly influenced by human presence. Rooms with high human activity showed a mean CFU/m<sup>3</sup> of  $1.1 \times 10^3$  (library cafeteria), while the Old Prints Storeroom, which is not publicly accessible, showed a mean CFU/m<sup>3</sup> of  $1.3 \times 10^2$  [36]. These results are in agreement with our observations since the Museum of King Jan III's Palace in Wilanow is a naturally ventilated indoor space with a high human presence.

### 3.2. Assessment of Bacterial Contamination of Air—Qualitative Analysis Using a Metagenomic Approach

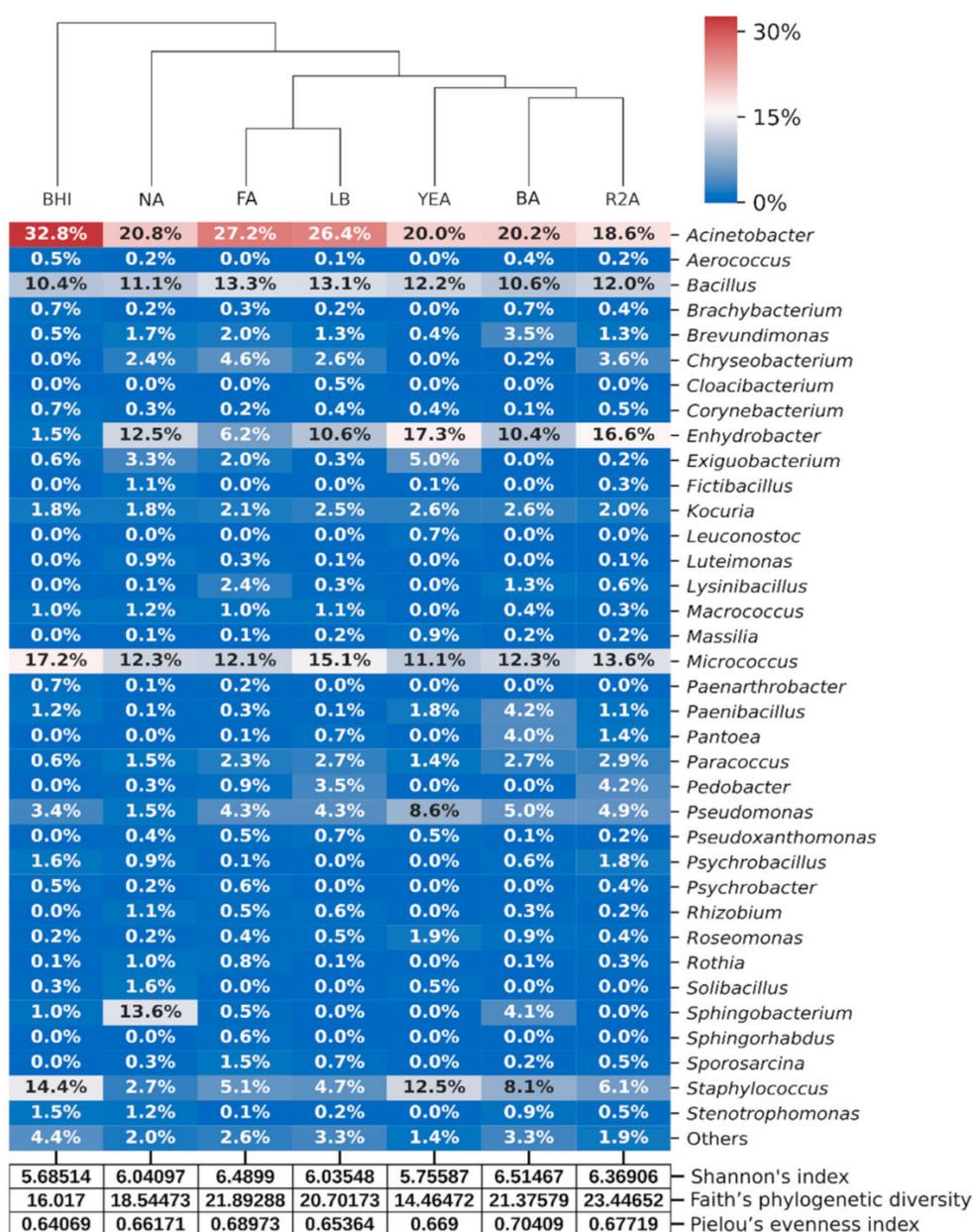
PCR amplicons were obtained using bacterial material collected from 1050 Petri dishes. Metagenomic analysis of seven samples (with each sample being the sum of bacteria cultured on a specific medium) revealed 130 bacterial genera. The number of bacterial genera obtained from each medium is presented in Table 1. Sequences that could not be classified at the genus level constituted between 1.3% (YEA medium) and 4.4% (BHI medium) of sequences obtained from each medium type and were assigned to the “others” group (Figure 3).

**Table 1.** Number of bacterial taxa recovered on each growth medium.

Taxonomic Rank	NA	LB	BHI	BA	FA	R2A	YEA
Genera	54	55	45	49	71	63	27
Families	30	30	29	30	38	38	21
Orders	17	16	15	16	20	21	12
Classes	6	5	5	6	5	7	5

NA—nutrient agar, LB—lysogeny broth, BHI—brain heart infusion agar, BA—Blickfeldt agar, FA—Frazier agar, R2A—Reasoner's 2A agar, YEA—yeast extract agar.

The relative abundance analysis revealed that all used media reported similar dominant genera, i.e., *Acinetobacter*, *Bacillus*, *Enhydrobacter*, *Micrococcus*, and *Staphylococcus* (Figure 3). Amongst them, *Acinetobacter*, *Enhydrobacter*, and *Staphylococcus* contain mainly species associated with common human skin microbiota, including several pathogens [37], whereas *Bacillus* and *Micrococcus* can be included in the large group of so-called environmental bacteria.



**Figure 3.** Cultivable bacteria taxonomy and their relative abundances. Heatmap with a dendrogram, depicting genera detected on the investigated media, and the following alpha-diversity indexes for each medium: Shannon’s index, Faith’s phylogenetic diversity, and Pielou’s evenness index. Only the genera that constituted at least 0.5%, abundance-wise, of all genera on at least one medium are shown on the heatmap. Genera accounting for less than 0.5% across all samples were assigned to the “others” group. NA—nutrient agar, LB—lysogeny broth, BHI—brain heart infusion agar, BA—Blickfeldt agar, FA—Frazier agar, R2A—Reasoner’s 2A agar, YEA—yeast extract agar.

While the dominant genera were similar on all media, the metabarcoding analysis showed that the total recovered bacterial taxa were different, depending on the type of medium. Shannon’s biodiversity and Pielou’s evenness indexes were similar for all media (Figure 3). On the other hand, Faith’s phylogenetic diversity index indicated significant differences between the tested samples. The lowest Faith’s phylogenetic diversity index was obtained for the YEA medium (14.46), while the R2A medium showed the highest value (23.45) (Figure 3). This result further supported the finding that rich media should not be used when trying to recover a high diversity of environmental microbiomes.

Based on the 16S rDNA amplicon analysis, we computed media combinations that would allow for the recovery of the highest bacterial diversity when sampling cultural heritage objects (Table 2). It was revealed that the FA medium enabled the growth of the most diverse community. It recovered 64.62% of cultivable bacterial genera detected on all tested media (Table 2). The addition of the R2A medium raised this value to 80.77% (by adding 21 new genera) (Table 2). Therefore, the combination of these two media could be used as a basis for microbiological assessments. The addition of other media subsequently increased the recovery rate, as shown in Table 2.

**Table 2.** Summary of the bacterial genera recovery using various media combinations.

Row No.	Media Combination *	Percent of All Genera Detected **	Genera Detected
1	FA	64.62%	<i>Acinetobacter, Aerococcus, Agrococcus, Algoriphagus, Amaricoccus, Arthrobacter, Aureimonas, Bacillus, Bosea, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Candidatus Paracaedibacter, Carnobacterium, Caulobacter, Chryseobacterium, Corynebacterium, Cupriavidus, Devosia, Dyadobacter, Enhydrobacter, Enterococcus, Exiguobacterium, Fictibacillus, Flavihumibacter, Flavobacterium, Glutamicibacter, Gordonia, Hydrogenophaga, Hymenobacter, Jeotgalicoccus, Kocuria, Kytococcus, Lactococcus, Luteimonas, Lysinibacillus, Lysobacter, Macroccoccus, Marmoricola, Massilia, Methylobacterium, Microbacterium, Micrococcus, Mycobacterium, Nakamurella, Nocardioideis, Novosphingobium, Oceanobacillus, Paenarthrobacter, Paenibacillus, Paeniglutamicibacter, Paenisporosarcina, Pantoea, Paracoccus, Pedobacter, Pseudomonas, Pseudorhodobacter, Pseudoxanthomonas, Psychrobacillus, Psychrobacter, Ralstonia, Rhizobacter, Rhizobium, Rhodobacter, Rhodococcus, Rhodoferax, Roseomonas, Rothia, Rummeliibacillus, Skermanella, Sphingobacterium, Sphingobium, Sphingomonas, Sphingopyxis, Sphingorhabdus, Sporosarcina, Staphylococcus, Stenotrophomonas, Streptococcus, Trichococcus, Truepera, Variovorax, Williamsia</i>
2	FA, R2A	80.77%	Row no. 1 + <i>Aeromicrobium, Agromyces, Cellulomonas, Cloacibacterium, Cohnella, Curtobacterium, Deinococcus, Dermacoccus, Dietzia, Escherichia-Shigella, Microvirga, Ornithinibacillus, Parasegetibacter, Planomicrobium, Porphyrobacter, Pseudarthrobacter, Pseudoclavibacter, Sanguibacter, Shinella, Solibacillus, Timonella</i>
3	FA, R2A, LB	89.23%	Row no. 2 + <i>Achromobacter, Clostridioides, Desemzia, Domibacillus, Enterobacter, Gracilibacillus, Kaistia, Leucobacter, Terribacillus, Vagococcus, Verticia</i>
4	FA, R2A, 0LB, NA	93.85%	Row no. 3 + <i>Aneurinibacillus, Bergeyella, Cellulosimicrobium, Nosocomiicoccus, Phenyllobacterium, Streptomyces</i>
5	FA, R2A, LB, NA, BA	96.92%	Row no. 4 + <i>Acidovorax, Lechevalieria, Ochrobactrum, Promicromonospora</i>
6	FA, R2A, LB, NA, BA, BHI	99.23%	Row no. 5 + <i>Dermabacter, Marinilactibacillus, Virgibacillus</i>
7	FA, R2A, LB, NA, BA, BHI, YEA	100%	Row no. 6 + <i>Leuconostoc</i>

\* NA—nutrient agar, LB—lysogeny broth, BHI—brain heart infusion agar, BA—Blickfeldt agar, FA—Frazier agar, R2A—Reasoner's 2A agar, YEA—yeast extract agar. \*\* The percentage of all genera detected is equal to the number of genera detected on a given medium or media combination, divided by the summarized number of unique genera grown on at least one tested medium.

This result can be used as a guideline for selecting optimal growth media for microbiological air pollution assessments. Usually, due to budget and human resources limitations, it is impossible

to use more than three different media during regular air contamination assessments in museums. Therefore, it is critical to establish reliable and comparable protocols (including medium selection) for classical analyses when employing microbial cultivation. In order to obtain the most comprehensive results, based on our findings, we suggest starting with the FA medium and then, depending on the available resources, adding other media in the following order: R2A, LB, NA, BA, BHI, and YEA.

### 3.3. Microbial Risk Assessment at the Museum Palace in Wilanow

#### 3.3.1. Bacteria Potentially Involved in the Biodeterioration and Biodegradation of Historical Art Pieces

Microbial activity plays an important role in the biodeterioration of objects of cultural heritage. This is primarily due to the extraordinary diversity of bacterial metabolism and the involvement of bacteria in biogeochemical processes that affect various surfaces. Lignocellulolytic microorganisms, found in most bacterial phyla, are capable of degrading lignocellulose, which is the main component of paper, plant-based textiles, and wood [38]. Animal-derived materials, such as wool, silk, or hide, are more resistant to degradation, as they are mainly made of keratin, which is a complex of densely packed amino acid chains. However, members of *Alcaligenes*, *Bacillus*, *Proteus*, *Pseudomonas*, and *Streptomyces* exhibiting keratolytic activity were found to be able to degrade those as well [39]. Successful colonization of historical objects by autotrophic bacteria results in the formation of syntrophic chains, in which one microorganism thrives on the metabolites produced by another species. This phenomenon further accelerates the disruption of a given material by indirect actions, such as acidification or increased water retention [40,41].

Previously performed studies highlighted the fact that the biodeterioration mechanisms occurring on historical art pieces, as well as their pace, are determined by the material the object is made of. The Museum Palace in Wilanow hosts a wide range of historical objects made of various materials, including textiles, stone, and wood. Therefore, each object may be subjected to different kinds of deteriorating microbial activity. In this study, we performed a general assessment of the microbial contamination of air at the Museum Palace in Wilanow to recognize the total microbial diversity and how these bacteria may potentially affect historical objects.

As mentioned above, amongst the identified bacteria, the three dominant taxa were *Bacillus*, *Micrococcus*, and *Pseudomonas*. These are heterotrophic bacteria that are often detected as secondary colonizers in biofilms, which are primarily produced by filamentous *Actinobacteria*. Interestingly, such biofilms were found to be responsible for damaging plaster, marble, and textiles [42]. While *Actinobacteria* build the foundation of these biofilms, by providing simpler organic carbon sources and retaining moisture, secondary colonizers introduce cellulolytic, hemicellulolytic, and keratolytic activity that further accelerates the biodegradation [42].

Although the dominant taxa potentially involved in biodegradation were discovered on all tested media, some other bacteria that may also be involved in the destruction of cultural heritage objects were detected exclusively on some media. Examples of such taxa were: (i) *Flavobacterium*, which was previously recognized to be marble degrader [43], was detected only on FA; (ii) members of the *Cytophagales* order, damaging wooden objects [44], were recovered on FA, R2A, and LB; (iii) *Sphingomonas*, recognized as an important biodeterioration agent, previously detected on old manuscripts, wooden sculptures, and mural paintings [45–47], was found on all media except BHI and YEA. Generally, it was observed that the majority of genera (including *Rhodococcus*, *Achromobacter*, *Oceanobacillus*, *Cellulomonas*, *Arthrobacter*, and *Streptomyces*) previously recognized as being involved in the biodeterioration of historical objects was detected only on selected non-rich media. This proves that the rich media commonly used for microbiological contamination assessments in museums generate strongly biased results.

### 3.3.2. Putative Bacterial Pathogens

While human-to-human transmission of pathogenic bacteria is strongly dependent on a pathogen's biology, many studies emphasize that the environment and its characteristics, such as indoor/outdoor location, active/passive ventilation, heating, humidity, and temperature, play an important role in pathogen viability [18,19,48]. Above all, direct human contact is the main driving factor for both viral and microbial infections. Museums and other institutions of cultural heritage can be considered as hot spots of increased biological air contamination due to their high human traffic. Therefore, their regular testing is of utmost importance.

Published studies investigating microbial air contamination usually focus on bacteria known to be etiological factors of respiratory diseases, such as *Mycobacterium tuberculosis*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, or members of the *Staphylococcus* genus [49–51]. In this study, we detected a relatively high number of representatives of the *Pseudomonas* and *Staphylococcus* genera (Figure 3). While *Pseudomonas* spp. host many species that are able to colonize a wide range of niches, *Staphylococcus* spp. are instead associated with a multitude of mammalian infections [52,53]. Human pathogens can also be found in both genera, including: (i) an opportunistic pathogen *P. aeruginosa*, which is a common multidrug-resistant bacterium that causes severe infections, especially in patients with cystic fibrosis [54]; (ii) *Pseudomonas mendocina*, an emerging pathogen that causes mainly nosocomial endocarditis and meningitis [55]; (iii) *Staphylococcus aureus* and *Staphylococcus epidermidis*, which are known to cause various skin and soft-tissue infections [56]. Moreover, amongst the detected genera, some can host opportunistic human pathogens, as is the case for the *Chryseobacterium* genus. Mukerji et al. described several serious infections caused by *Chryseobacterium indologenes*, a bacterium that is usually found in soil and water [57].

In order to quantify the applicability of the used growth media for the detection of human pathogens in museums, we conducted a systematic literature review, as described in Section 2.5, to identify genera that potentially contain human pathogens. This analysis revealed that 64 out of 130 genera detected in this study contained bacterial species reported to be human pathogens (Table 3). Despite the very high contribution of genera containing human pathogens—49.23%, it should be noted that most of them are opportunistic pathogens, which are mostly dangerous, specifically for immunocompromised individuals. Table 3 shows that the FA and R2A media detected up to 46 genera containing human pathogens, while NA—42, LB—40, BHI and BA—37, and YEA only 19. These results were in line with general genus recovery rates presented in Table 2, showing that the media (FA and R2A) capable of recovering the highest number of different bacterial genera were also suitable for the detection of species that are potentially pathogenic to humans.

It is also important to highlight that one of the dominant genera found in the Museum of King John III's Palace at Wilanow, namely, *Acinetobacter*, contains two clinically important pathogenic species, namely, *A. baumannii* and *A. lwoffii*, which are both often characterized by their ability to form complex biofilms that reduce their sensitivity to many antibacterial agents, such as antibiotics [58,59]. Although *A. baumannii* is rarely found outside hospitals, it is still possible that a museum microclimate, with its stable temperature and humidity, may provide suitable conditions for its development.

**Table 3.** Summary of the bacterial genera containing human pathogens that were recovered on various media.

Medium	No. of Genera	Genera Containing Human Pathogens
FA	46	<i>Acinetobacter, Aerococcus, Agrococcus, Arthrobacter, Aureimonas, Bacillus, Bosea, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Caulobacter, Chryseobacterium, Corynebacterium, Cupriavidus, Devosia, Enterococcus, Exiguobacterium, Flavobacterium, Gordonia, Kocuria, Kytococcus, Lactococcus, Macroccoccus, Massilia, Methylobacterium, Microbacterium, Micrococcus, Mycobacterium, Novosphingobium, Paenibacillus, Pantoea, Paracoccus, Pseudomonas, Psychrobacter, Ralstonia, Rhizobium, Rhodococcus, Roseomonas, Rothia, Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptococcus, Williamsia</i>
R2A	46	<i>Acinetobacter, Aerococcus, Agrococcus, Arthrobacter, Aureimonas, Bacillus, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Caulobacter, Cellulomonas, Chryseobacterium, Corynebacterium, Curtobacterium, Dermacoccus, Dietzia, Enterococcus, Escherichia-Shigella, Exiguobacterium, Gordonia, Kocuria, Kytococcus, Lactococcus, Macroccoccus, Massilia, Methylobacterium, Microbacterium, Micrococcus, Mycobacterium, Ornithinibacillus, Paenibacillus, Pantoea, Paracoccus, Pseudoclavibacter, Pseudomonas, Psychrobacter, Ralstonia, Rhizobium, Rhodococcus, Roseomonas, Rothia, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptococcus</i>
NA	42	<i>Acinetobacter, Aerococcus, Arthrobacter, Aureimonas, Bacillus, Bergeyella, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Cellulosimicrobium, Chryseobacterium, Clostridioides, Corynebacterium, Curtobacterium, Dermacoccus, Dietzia, Enterococcus, Exiguobacterium, Gordonia, Kocuria, Kytococcus, Macroccoccus, Massilia, Microbacterium, Micrococcus, Novosphingobium, Paenibacillus, Paracoccus, Pseudomonas, Psychrobacter, Ralstonia, Rhizobium, Rhodococcus, Roseomonas, Rothia, Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptococcus, Streptomyces</i>
LB	40	<i>Achromobacter, Acinetobacter, Aerococcus, Agrococcus, Aureimonas, Bacillus, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Chryseobacterium, Clostridioides, Corynebacterium, Cupriavidus, Dietzia, Enterobacter, Enterococcus, Escherichia-Shigella, Exiguobacterium, Kocuria, Kytococcus, Macroccoccus, Massilia, Microbacterium, Micrococcus, Paenibacillus, Pantoea, Paracoccus, Pseudomonas, Psychrobacter, Ralstonia, Rhizobium, Rhodococcus, Roseomonas, Rothia, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptococcus, Vagococcus</i>
BHI	37	<i>Achromobacter, Acinetobacter, Aerococcus, Aureimonas, Bacillus, Brachybacterium, Brevibacterium, Brevundimonas, Cellulosimicrobium, Corynebacterium, Curtobacterium, Dermabacter, Dermacoccus, Dietzia, Enterobacter, Enterococcus, Exiguobacterium, Kocuria, Kytococcus, Macroccoccus, Massilia, Microbacterium, Micrococcus, Novosphingobium, Paenibacillus, Pantoea, Paracoccus, Pseudomonas, Psychrobacter, Ralstonia, Roseomonas, Rothia, Sphingobacterium, Staphylococcus, Stenotrophomonas, Streptococcus, Streptomyces</i>
BA	37	<i>Acidovorax, Acinetobacter, Aerococcus, Arthrobacter, Aureimonas, Bacillus, Brachybacterium, Brevibacillus, Brevibacterium, Brevundimonas, Cellulomonas, Chryseobacterium, Corynebacterium, Curtobacterium, Dietzia, Enterococcus, Kocuria, Macroccoccus, Massilia, Microbacterium, Micrococcus, Mycobacterium, Ochrobactrum, Paenibacillus, Pantoea, Paracoccus, Pseudomonas, Ralstonia, Rhizobium, Rhodococcus, Roseomonas, Rothia, Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas, Streptococcus</i>
YEA	19	<i>Acinetobacter, Bacillus, Brachybacterium, Brevibacterium, Brevundimonas, Corynebacterium, Dietzia, Exiguobacterium, Kocuria, Leuconostoc, Massilia, Microbacterium, Micrococcus, Paenibacillus, Paracoccus, Pseudomonas, Ralstonia, Roseomonas, Staphylococcus</i>

NA—nutrient agar, LB—lysogeny broth, BHI—brain heart infusion agar, BA—Blickfeldt agar, FA—Frazier agar, R2A—Reasoner's 2A agar, YEA—yeast extract agar.

#### 4. Conclusions

The results obtained in this work emphasize the need to further develop standardized operational protocols for microbial air pollution assessment of objects of cultural heritage. The NGS analysis demonstrated that the selection of an appropriate medium played a crucial role in assessing the diversity of cultivable bacterial communities.

The results obtained in this work clearly show that rich media, which are currently the most commonly used type of media for the assessment of microbial air contamination, produced heavily biased results. The use of rich media promoted fast-growing bacteria, which in turn, led to the inhibition of the growth of other species. This may prevent the detection of the biodeteriorating and pathogenic bacteria present in a museum. To solve this problem, we combined currently used classical microbiology methods with a novel NGS-based approach. Our results showed that Frazier medium, which is a type of minimal medium, led to the recovery of the most diverse bacterial community. Therefore, it was suggested that rich media should be replaced by FA (preferentially in combination with R2A) medium in protocols applied in museums. This combination would allow for the detection of more bacterial species in general, as well as specifically human pathogens, and therefore lead to a better understanding of the biodeteriorating and pathogenic microflora that are present in museums.

**Supplementary Materials:** The following is available online at <http://www.mdpi.com/2076-3417/10/20/7128/s1>, Table S1: *p*-values of the pairwise comparisons between growth media CFU/m<sup>3</sup> counts.

**Author Contributions:** Conceptualization, M.D. (Mikolaj Dziurzynski), M.D. (Magdalena Dyda), and L.D.; data curation, M.D. (Mikolaj Dziurzynski); formal analysis, M.D. (Mikolaj Dziurzynski), M.D. (Magdalena Dyda), and L.D.; funding acquisition, M.D. (Magdalena Dyda) and A.L.; investigation, M.D. (Mikolaj Dziurzynski), M.D. (Magdalena Dyda), A.S., P.D., and L.D.; methodology, M.D. (Mikolaj Dziurzynski), M.D. (Magdalena Dyda), and L.D.; project administration, M.D. (Magdalena Dyda) and A.L.; resources, M.D. (Magdalena Dyda), A.L., and L.D.; software, M.D. (Mikolaj Dziurzynski); supervision, M.D. (Magdalena Dyda) and L.D.; validation, M.D. (Mikolaj Dziurzynski) and L.D.; visualization, M.D. (Mikolaj Dziurzynski) and L.D.; writing—original draft, M.D. (Mikolaj Dziurzynski), K.C., and L.D.; writing—review and editing, M.D. (Mikolaj Dziurzynski), K.C., M.D. (Magdalena Dyda), A.L., and L.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the “Intelligent system for measuring environmental parameters with built-in predictive models for the protection of cultural heritage” project, which is co-financed by the European Regional Development Fund as part of the European Regional Development Fund: Priority axis I “Use of research and development activities in the economy”, measure 1.2 “R&D activities of enterprises”, Regional Operational Programme of the Mazowieckie Voivodeship for the years 2014–2020, agreement no. RPMA.01.02.00-14-5780/16-00.

**Acknowledgments:** The authors are grateful to Antonina Ignatenko and Damian Kakietek for the technical support regarding the microbiological sampling. Special thanks to Michalina Grzegorzczak for the efficient organization of the measurements in the Museum.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. Wells, W.F. *Airborne Contagion and Air Hygiene. An Ecological Study of Droplet Infections*; Harvard University Press: Cambridge, UK, 1955.
2. Górny, R.L.; Cyprowski, M.; Ławniczek-Wałczyk, A.; Gołofit-Szymczak, M.; Zapór, L. Biohazards in the Indoor Environment—A Role for Threshold Limit Values in Exposure Assessment. In *The Management of Indoor Air Quality*; Dudzinska, M.R., Ed.; CRC Press: Boca Raton, FL, USA, 2011; ISBN 9780415672665.
3. Scaltriti, S.; Cencetti, S.; Rovesti, S.; Marchesi, I.; Bargellini, A.; Borella, P. Risk factors for particulate and microbial contamination of air in operating theatres. *J. Hosp. Infect.* **2007**, *66*, 320–326. [[CrossRef](#)] [[PubMed](#)]
4. Napoli, C.; Marcotrigiano, V.; Montagna, M.T. Air sampling procedures to evaluate microbial contamination: A comparison between active and passive methods in operating theatres. *BMC Public Health* **2012**, *12*, 594. [[CrossRef](#)] [[PubMed](#)]
5. Hayleeyesus, S.F.; Manaye, A.M. Microbiological quality of indoor air in university libraries. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, S312–S317. [[CrossRef](#)] [[PubMed](#)]

6. Kovats, N.; Horvath, E.; Jancsek-Turoczi, B.; Hoffer, A.; Gelencser, A.; Urban, P.; Kiss, I.E.; Bihari, Z.; Fekete, C. Microbiological characterization of stable resuspended dust. *Int. J. Occup. Med. Environ. Health* **2016**, *29*, 375–380. [[CrossRef](#)]
7. Valeriani, F.; Cianfanelli, C.; Gianfranceschi, G.; Santucci, S.; Romano Spica, V.; Mucci, N. Monitoring biodiversity in libraries: A pilot study and perspectives for indoor air quality. *J. Prev. Med. Hyg.* **2017**, *58*, E238–E251.
8. Taberlet, P.; Coissac, E.; Pompanon, F.; Brochmann, C.; Willerslev, E. Towards next-generation biodiversity assessment using DNA metabarcoding. *Mol. Ecol.* **2012**, *21*, 2045–2050. [[CrossRef](#)]
9. Tarsitani, G.; Moroni, C.; Cappitelli, F.; Pasquariello, G.; Maggi, O. Microbiological analysis of surfaces of Leonardo da Vinci's Atlantic Codex: Biodeterioration risk. *Int. J. Microbiol.* **2014**. [[CrossRef](#)]
10. Pinar, G.; Krakova, L.; Pangallo, D.; Piombino-Mascalì, D.; Maixner, F.; Zink, A.; Sterflinger, K. Halophilic bacteria are colonizing the exhibition areas of the Capuchin Catacombs in Palermo, Italy. *Extremophiles* **2014**, *18*, 677–691. [[CrossRef](#)]
11. Saiz-Jimenez, C.; Miller, A.Z.; Martin-Sanchez, P.M.; Hernandez-Marine, M. Uncovering the origin of the black stains in Lascaux Cave in France. *Environ. Microbiol.* **2012**, *14*, 3220–3231. [[CrossRef](#)]
12. Kusumi, A.; Li, X.; Osuga, Y.; Kawashima, A.; Gu, J.-D.; Nasu, M.; Katayama, Y. Bacterial communities in pigmented biofilms formed on the sandstone bas-relief walls of the Bayon Temple, Angkor Thom, Cambodia. *Microbes Environ.* **2013**, *28*, 422–431. [[CrossRef](#)]
13. Osimani, A.; Aquilanti, L.; Tavoletti, S.; Clementi, F. Microbiological monitoring of air quality in a university canteen: An 11-year report. *Environ. Monit. Assess.* **2013**, *185*, 4765–4774. [[CrossRef](#)] [[PubMed](#)]
14. Giovannoni, S.J.; Britschgi, T.B.; Moyer, C.L.; Field, K.G. Genetic diversity in Sargasso Sea bacterioplankton. *Nature* **1990**, *345*, 60–63. [[CrossRef](#)] [[PubMed](#)]
15. Ward, D.M.; Weller, R.; Bateson, M.M. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* **1990**, *345*, 63–65. [[CrossRef](#)] [[PubMed](#)]
16. Hugenholtz, P. Exploring prokaryotic diversity in the genomic era. *Genome Biol.* **2002**, *3*, reviews0003-1. [[CrossRef](#)]
17. Mirhoseini, S.H.; Nikaeen, M.; Khanahmad, H.; Hassanzadeh, A. Occurrence of airborne vancomycin- and gentamicin-resistant bacteria in various hospital wards in Isfahan, Iran. *Adv. Biomed. Res.* **2016**, *5*, 143. [[CrossRef](#)]
18. Herfst, S.; Bohringer, M.; Karo, B.; Lawrence, P.; Lewis, N.S.; Mina, M.J.; Russell, C.J.; Steel, J.; de Swart, R.L.; Menge, C. Drivers of airborne human-to-human pathogen transmission. *Curr. Opin. Virol.* **2017**, *22*, 22–29. [[CrossRef](#)]
19. Luongo, J.C.; Fennelly, K.P.; Keen, J.A.; Zhai, Z.J.; Jones, B.W.; Miller, S.L. Role of mechanical ventilation in the airborne transmission of infectious agents in buildings. *Indoor Air* **2016**, *26*, 666–678. [[CrossRef](#)]
20. Dyda, M.; Decewicz, P.; Romaniuk, K. International Biodeterioration & Biodegradation Application of metagenomic methods for selection of an optimal growth medium for bacterial diversity analysis of microbiocenoses on historical stone surfaces. *Int. Biodeterior. Biodegrad.* **2017**, 2–10. [[CrossRef](#)]
21. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [[CrossRef](#)]
22. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [[CrossRef](#)]
23. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [[CrossRef](#)] [[PubMed](#)]
24. Callahan, B.J.; McMurdie, P.J.; Holmes, S.P. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J.* **2017**, *11*, 2639–2643. [[CrossRef](#)] [[PubMed](#)]
25. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glockner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, 590–596. [[CrossRef](#)] [[PubMed](#)]
26. Hunter, J.D. Matplotlib: A 2D graphics environment. *Comput. Sci. Eng.* **2007**, *9*, 90–95. [[CrossRef](#)]
27. Waskom, M.; Botvinnik, O.; Ostblom, J.; Saulius, L.; Hobson, P. Mwaskom/Seaborn:v0.10.0 2020. Available online: <https://github.com/mwaskom/seaborn/tree/v0.9.0> (accessed on 11 October 2020).

28. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2017.
29. RStudio Team. *RStudio: Integrated Development Environment for R*; RStudio, PBC: Boston, MA, USA, 2015.
30. Grisoli, P.; Albertoni, M.; Rodolfi, M. Application of airborne microorganism indexes in offices, gyms, and libraries. *Appl. Sci.* **2019**, *9*, 1101. [[CrossRef](#)]
31. Godish, D.R.; Godish, T.J. Relationship between sampling duration and concentration of culturable airborne mould and bacteria on selected culture media. *J. Appl. Microbiol.* **2007**, *102*, 1479–1484. [[CrossRef](#)]
32. Dacarro, C.; Picco, A.M.; Grisoli, P.; Rodolfi, M. Determination of aerial microbiological contamination in scholastic sports environments. *J. Appl. Microbiol.* **2003**, *95*, 904–912. [[CrossRef](#)]
33. Hyvärinen, A.M.; Martikainen, P.J.; Nevalainen, A.I. Suitability of poor medium in counting total viable airborne bacteria. *Grana* **1991**, *30*, 414–417. [[CrossRef](#)]
34. Brągoszewska, E.; Bogacka, M.; Pikoń, K. Efficiency and eco-costs of air purifiers in terms of improving microbiological indoor air quality in dwellings—A case study. *Atmosphere* **2019**, *10*, 742. [[CrossRef](#)]
35. Brągoszewska, E.; Biedroń, I.; Kozielska, B.; Pastuszka, J.S. Microbiological indoor air quality in an office building in Gliwice, Poland: Analysis of the case study. *Air Qual. Atmos. Heal.* **2018**, *11*, 729–740. [[CrossRef](#)]
36. Kalwasińska, A.; Burkowska, A.; Wilk, I. Microbial air contamination in indoor environment of a university library. *Ann. Agric. Environ. Med.* **2012**, *19*, 25–29. [[PubMed](#)]
37. Castelino, M.; Eyre, S.; Moat, J.; Fox, G.; Martin, P.; Ho, P.; Upton, M.; Barton, A. Optimisation of methods for bacterial skin microbiome investigation: Primer selection and comparison of the 454 versus MiSeq platform. *BMC Microbiol.* **2017**, *17*, 23. [[CrossRef](#)] [[PubMed](#)]
38. Mazzoli, R.; Giuffrida, M.G.; Pessione, E. Back to the past: “Find the guilty bug-microorganisms involved in the biodeterioration of archeological and historical artifacts”. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 6393–6407. [[CrossRef](#)] [[PubMed](#)]
39. Szostak-Kotowa, J. Biodeterioration of textiles. *Int. Biodeterior. Biodegradation* **2004**, *53*, 165–170. [[CrossRef](#)]
40. Sterflinger, K.; Piñar, G. Microbial deterioration of cultural heritage and works of art—tilting at windmills? *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9637–9646. [[CrossRef](#)]
41. Caselli, E.; Pancaldi, S.; Baldisserotto, C.; Petrucci, F.; Impallaria, A.; Volpe, L.; D’Accolti, M.; Soffritti, I.; Coccagna, M.; Sassu, G.; et al. Characterization of biodegradation in a 17th century easel painting and potential for a biological approach. *PLoS ONE* **2018**, *13*, e0207630. [[CrossRef](#)]
42. Saarela, M.; Alakomi, H.-L.; Suihko, M.-L.; Maunuksela, L.; Raaska, L.; Mattila-Sandholm, T. Heterotrophic microorganisms in air and biofilm samples from Roman catacombs, with special emphasis on actinobacteria and fungi. *Int. Biodeterior. Biodegrad.* **2004**, *54*, 27–37. [[CrossRef](#)]
43. Sorlini, C.; Zanardini, E.; Albo, S.; Praderio, G.; Cariati, F.; Bruni, S. Research on chromatic alterations of marbles from the fountain of Villa Litta (Lainate, Milan). *Int. Biodeterior. Biodegrad.* **1994**, *33*, 153–164. [[CrossRef](#)]
44. Nilsson, T.; Björdal, C.; Fällman, E. Culturing erosion bacteria: Procedures for obtaining purer cultures and pure strains. *Int. Biodeterior. Biodegrad.* **2008**, *61*, 17–23. [[CrossRef](#)]
45. Palla, F.; Mancuso, F.P.; Billeci, N. Multiple approaches to identify bacteria in archaeological waterlogged wood. *J. Cult. Herit.* **2013**, *14*, e61–e64. [[CrossRef](#)]
46. Pasquarella, C.; Balocco, C.; Pasquariello, G.; Petrone, G.; Sacconi, 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.* **2015**, *536*, 557–567. [[CrossRef](#)] [[PubMed](#)]
47. Heyrman, J.; Swings, J. 16S rDNA sequence analysis of bacterial isolates from biodeteriorated mural paintings in the Servilia tomb (Necropolis of Carmona, Seville, Spain). *Syst. Appl. Microbiol.* **2001**, *24*, 417–422. [[CrossRef](#)] [[PubMed](#)]
48. Tang, J.W. The effect of environmental parameters on the survival of airborne infectious agents. *J. R. Soc. Interface* **2009**, *6* (Suppl. 6), S737–S746. [[CrossRef](#)] [[PubMed](#)]
49. Nguyen, T.M.N.; Illef, D.; Jarraud, S.; Rouil, L.; Campese, C.; Che, D.; Haeghebaert, S.; Ganiayre, F.; Marcel, F.; Etienne, J.; et al. A community-wide outbreak of legionnaires disease linked to industrial cooling towers—how far can contaminated aerosols spread? *J. Infect. Dis.* **2006**, *193*, 102–111. [[CrossRef](#)] [[PubMed](#)]
50. Fronczek, C.F.; Yoon, J.-Y. Biosensors for monitoring airborne pathogens. *J. Lab. Autom.* **2015**, *20*, 390–410. [[CrossRef](#)]

51. Nuermberger, E.; Bishai, W.R.; Grosset, J.H. Latent tuberculosis infection. *Semin. Respir. Crit. Care Med.* **2004**, *25*, 317–336. [[CrossRef](#)]
52. Jun, S.-R.; Wassenaar, T.M.; Nookaew, I.; Hauser, L.; Wanchai, V.; Land, M.; Timm, C.M.; Lu, T.-Y.S.; Schadt, C.W.; Doktycz, M.J.; et al. Diversity of *Pseudomonas* genomes, including populus-associated isolates, as revealed by comparative genome analysis. *Appl. Environ. Microbiol.* **2016**, *82*, 375–383. [[CrossRef](#)]
53. Haag, A.F.; Fitzgerald, J.R.; Penadés, J.R. *Staphylococcus aureus* in animals. *Microbiol. Spectr.* **2019**, *7*. [[CrossRef](#)]
54. Stefani, S.; Campana, S.; Cariani, L.; Carnovale, V.; Colombo, C.; Lleo, M.M.; Iula, V.D.; Minicucci, L.; Morelli, P.; Pizzamiglio, G.; et al. Relevance of multidrug-resistant *Pseudomonas aeruginosa* infections in cystic fibrosis. *Int. J. Med. Microbiol.* **2017**, *307*, 353–362. [[CrossRef](#)]
55. Gani, M.; Rao, S.; Miller, M.; Scoular, S. *Pseudomonas mendocina* bacteremia: A case study and review of literature. *Am. J. Case Rep.* **2019**, *20*, 453–458. [[CrossRef](#)]
56. Natsis, N.E.; Cohen, P.R. Coagulase-negative *Staphylococcus* skin and soft tissue infections. *Am. J. Clin. Dermatol.* **2018**, *19*, 671–677. [[CrossRef](#)] [[PubMed](#)]
57. Mukerji, R.; Kakarala, R.; Smith, S.J.; Kusz, H.G. *Chryseobacterium indologenes*: An emerging infection in the USA. *BMJ Case Rep.* **2016**. [[CrossRef](#)] [[PubMed](#)]
58. Antunes, L.C.S.; Visca, P.; Towner, K.J. *Acinetobacter baumannii*: Evolution of a global pathogen. *Pathog. Dis.* **2014**, *71*, 292–301. [[CrossRef](#)] [[PubMed](#)]
59. Pour, N.K.; Dusane, D.H.; Dhakephalkar, P.K.; Zamin, F.R.; Zinjarde, S.S.; Chopade, B.A. Biofilm formation by *Acinetobacter baumannii* strains isolated from urinary tract infection and urinary catheters. *FEMS Immunol. Med. Microbiol.* **2011**, *62*, 328–338. [[CrossRef](#)] [[PubMed](#)]

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).