Microbiological diversity and microbial related risks at Wawel Royal Castle in Krakow, Poland

Pyzik A.[1,2,*], Dyda M.[1,2], Wiłkojć E.[3], Winiewicz-Wolska J.[3]

[1]Research and Development for Life Sciences Ltd.; Miecznikowa 1/5a, 02-096 Warsaw, Poland
[2]Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland
[3]Wawel Royal Castle, Wawel 5, 31-001 Krakow, Poland

*corresponding author: e-mail: a.pyzik@rdls.pl

Abstract
Microorganisms are known to colonize and modify various environments, including cultural heritage buildings. Anthropogenic indoor environments often provide specific conditions for microbial growth e.g. microclimatic parameters. These conditions are potentially easy to control thus it is advisable to characterize and monitor bacterial and fungal communities in indoor habitats. Microbiological pollution in museums is particularly important because bacteria and fungi contribute to the deterioration of cultural heritage objects and may have a negative impact on human health. In our study we performed microbiological analysis of Wawel Royal Castle in Krakow, which is one of the greatest Polish national heritage sites and was visited by over 1.4 million tourists in 2017. There are large and valuable collections of objects related to the Polish rulers e.g. rare tapestries and paintings. Microbiome analysis was done by culture-dependent experiments for I) airborne microorganisms (50 litres of air) sampled onto various types of agar media, as well as culture-independent analysis of II) airborne microorganisms (12000 litres of air) sampled onto saline, and III) microbes from dust from historical surfaces. Microbiological diversity was determined by analysis of deeply sequenced amplicons covering hypervariable regions V3-V4 of 16S rDNA gene and ITS2 region located between 5.8S and 26S Rdna especially when in a complex interspecies consortium. Usually, environmental microbial communities are formed by many taxonomic groups of organisms, which are often unculturable in the laboratory conditions. The knowledge about the structure of microbial communities in cultural heritage may lead to preventive measures before the biodetorioration process of valuable historical objects
Keywords: cultural heritage, microbiological diversity, high-throughput sequencing

1. Introduction

Cultural heritage buildings and objects are heterogeneous habitats, which could be colonized by wide spectrum of organisms such as *Bacteria*, *Archaea*, *Cyanobacteria*, *Algae*, *Fungi*, and *Lichens* [Di Carlo et al., 2017]. One of the most important matters is their impact on the degradation (biodetorioration) of artworks and on human health. It seems that bacteria and fungi have the greatest impact as they are relatively easily transferred between surfaces, air and water. Moreover, they have usually high growth rates, tolerate a wide range of pH, temperature and often can degrade various compounds, which are unavailable for other organisms. Both bacteria and fungi easily adjust to unfavorable environmental conditions,

2. Methods

Samples were collected in an exhibition room, with heavy tourist traffic, located on the second floor of Wawel Royal Castle in Krakow. In October the following three types of samples were obtained: (1) 50 litres of air sampled onto various types of agar media (with the use of MAS-100 Eco® air sampler (Merck)).; (2) 12000 litres of air sampled onto saline and (3) dust from surface of historical furniture. The latter two samples were subjected for direct DNA isolation while the former one was used for the cultivation of airborne microorganisms. Bacteria and fungi were incubated for 48 h at 37°C and 168 h at 26°C, respectively. After the cultivation, the DNA was isolated from all grown bacterial and fungal colonies. For all types of samples, extraction of DNA was performed with the use of FastDNA™ SPIN Kit for Feces (MP Biomedica) and FastPrep® bead beater (MP Biomedica). Microbiological diversity was determined by the analysis of deeply sequenced amplicons covering hypervariable regions V3V4 of 16S rDNA gene and ITS2 region located between 5.8S and 26S rDNA.

3. Results

Our results showed that the highest diversity at genera level was obtain for culture-independent analysis of dust microbiome followed by culture-independent analysis of airborne microbiome (12000L of air) and culture-dependent analysis of airborne microbiome (50L of air) cultivated on various agar media (Table. 1). Culture-independent analysis of microbial diversity showed that dust microbiome was 6-fold (bacteria) and 8-fold (fungi) richer then airborne microbiome. At genera level, 120 taxonomic groups of bacteria and 67 of fungi were detected in both dust and air microbiome analyzed by direct methodology. In the case of the same type of sample, namely air, there were approximately 3-times greater abundance of microorganisms analyzed by culture-independent method than culture-dependent. There were

37 genera of bacteria and 18 genera of fungi common for both types of sampled air (Table 1).

Table 1. Diversity of microorganisms (at genera level) based on sample type and methodology used.

	Number of identified	
	Bacteria	Fungi
air - 50 L (c-d)	49	23
air - 12000 L (c-i)	131	73
dust (c-i)	791	593
common for the same sample type- air (50L c-d vs 12000L c-i)	37	18
common for methodology type - c-i (dust vs 12000L of air)	120	67

c-d – culture-dependent; c-i – culture-independent

Overall for each of the analyzed samples, we detected up to 12 dominant taxonomic groups of microorganisms with relative abundance greater than 2% of community structure. Despite of the fact that some of microorganisms overlapped between analyzed sample pairs (50 L vs 12000 L of air or dust vs 12000 L of air), the dominant microbes were different in each sample (Fig. 1 and Fig. 2). In the case of airborne bacteria, the most relatively abundant genera cultivated on agar plates were Staphylococcus (23%), Micrococcus (18%), and Bacillus (18%), while direct isolation of microorganisms revealed evident predominance of Pseudomonas (30%) (Fig. 1). Relative abundance of dominant fungi showed supremacy of Penicillium (~32%) and Cladosporium (~18%) in both air microbiomes, yet cultivation overrepresented Trichoderma (39%) while in the directly isolated airborne microorganisms there was high abundance of Fusarium (12%) (Fig. 2).

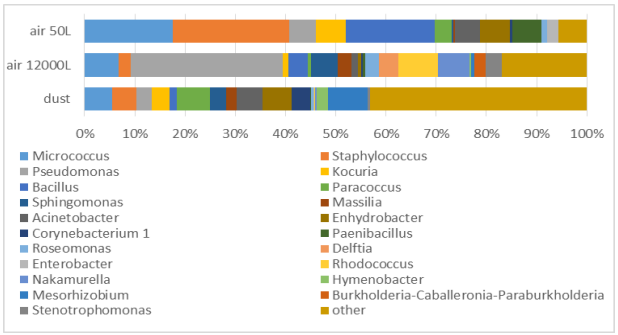

Figure 1. Relative abundance of bacteria based on bacterial V3V4 16S rDNA gene fragment. The bar chart shows by default the relative abundance of genera with abundance greater than 2% in at least one variant.

Comparison of microbial diversity of directly isolated microorganisms showed that dominant taxonomic groups were very different for dust and air samples. However, in both types of samples there were numerous low abundant (<2%) microorganisms which cumulatively account for 46% and 33% in dust and 23% and 20% in air, bacteria and fungi respectively. The most predominant bacteria in dust were *Mesorhizobium* (8%) and *Paracoccus* (7%) while fungal communities were dominated by *Alternaria* (22%), family *Didymellaceae* (11%), order *Capnodiales* (11%), and *Aureobasidium* (7%).

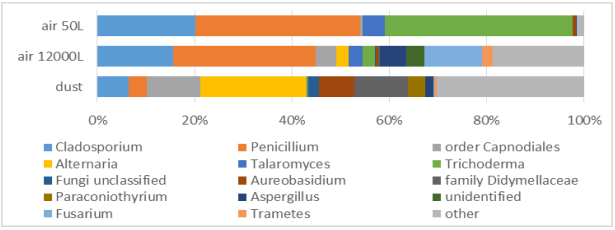

Figure 2. Relative abundance of fungi based on fungal ITS2 region. The bar chart shows by default the relative abundance of genera with abundance greater than 2% in at least one variant. Sequences that were not assigned at the family level were named in accordance with the lowest available taxonomy.

4. Discussion

The results demonstrated that different type of samples and methodology used, can deliver different insight into the diversity of microbial communities involved in the biodetorioration of cultural heritage or those that can affect human health e. g. *Penicillium* or *Staphylococcus*. Application of various media increase the probability of obtaining broad range of microorganisms, however culture-independent techniques should be preferentially applied as they allows characterization of native microbial communities [Laiz et al., 2003; Dyda et al., 2017].

It seems that culture-dependent methods could be more reliably applied for the diversity of airborne fungi than bacteria. Nevertheless, fungi should be more intensively studied in museums as large amount of sequences specific for dust sample were classified only for higher levels of taxonomical categories. Furthermore, diversity analysis of dust and air microbiomes suggests that there is a higher probability that airborne microorganisms are deposited on the surface of historical object rather than dust microbes are mobilized into the air.

Acknowledgements

This work was supported in the framework of the "Wawel – heritage for the future" ("Wawel-dziedzictwo dla przyszłości") project of VIII Priority Axis Protection of Cultural Heritage and development of cultural resources financed by the European Regional Development Fund as part of the Operational Program Infrastructure and Environment 2014-2020

References

Di Carlo, E., Barresi, G., & Palla, F. (2017). Biodeterioration. In Biotechnology and Conservation of Cultural Heritage (pp. 1-30). Springer, Cham

Laiz, L., Piñar, G., Lubitz, W., & Saiz-Jimenez, C. (2003). Monitoring the colonization of monuments by bacteria: cultivation versus molecular methods. Environmental Microbiology, 5(1), 72-74

Dyda, M., Decewicz, P., Romaniuk, K., Wojcieszak, M., Sklodowska, A., Dziewit, L., ... & Laudy, A. (2018). Application of metagenomic methods for selection of an optimal growth medium for bacterial diversity analysis of microbiocenoses on historical stone surfaces. International Biodeterioration & Biodegradation, 131, 2-10.