# Selection of an optimal growth medium for bacterial biodiversity analysis in the biocenoses observed on historical stone surfaces. Example for application of metagenomics methods.

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## Introduction

Identification of microorganisms on the surfaces of historical objects is traditionally based on the classical microbiology techniques. Due to obstacles in determining growth requirements of each of the isolated microbial species, cultivation methods only to a limited extent allow the investigation of the microbial biodiversity occurring in the environment. Therefore, there is a need for determination, which of the known commonly used microbiological media enable comparable assessment of the biodiversity of bacteria found on the historical objects. In the present study, we have assessed the composition of the microbial community *in situ* in biocenosis on historical sandstone Pergola with microbial diversity observed after cultivation of samples on different agar plates.

## Aim

**The aim of the study was to select microbial media, the use of which will allow the most accurate determination of the proportion and biodiversity of bacteria present in biocenoses on historical stone objects.**

## Results

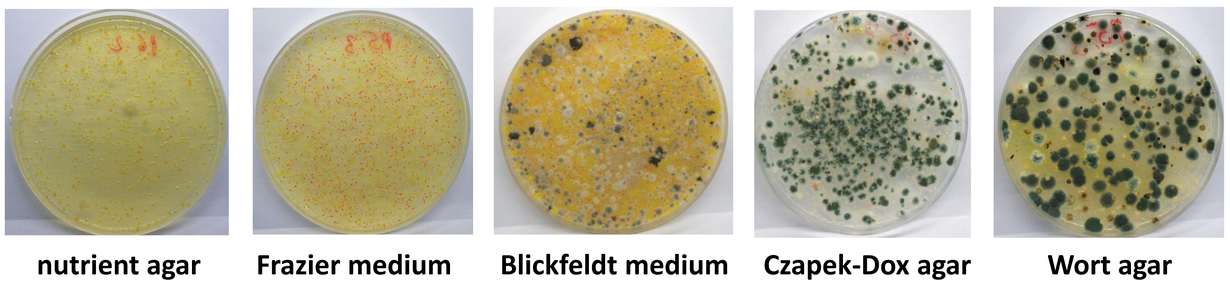



Fig. 2. Examples of microbial growth observed on different agar plates.

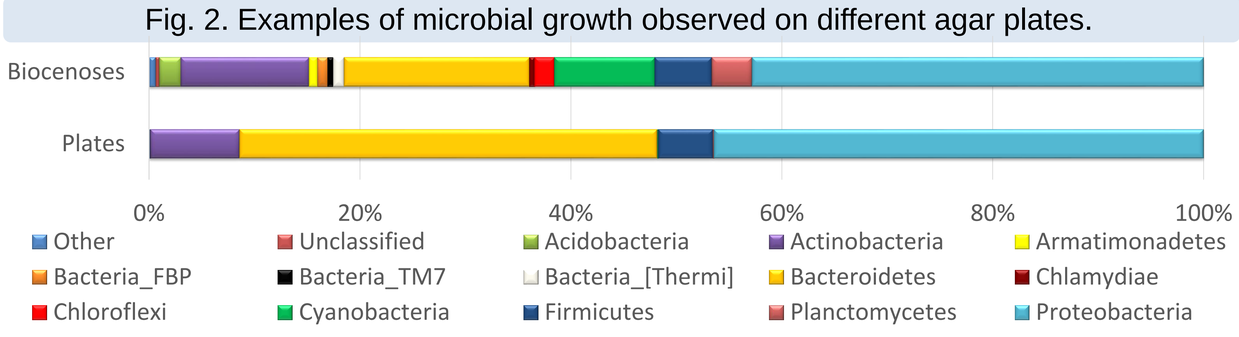
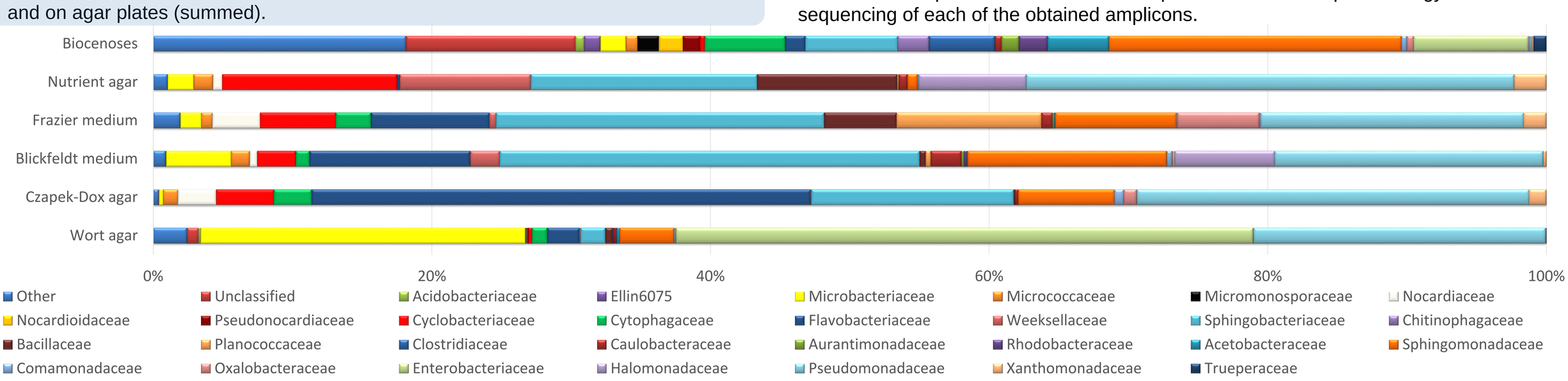



Fig. 3. Bacterial biodiversity based on 16S bacterial rRNA gene analysis. Data for bacterial phyla constituted more than 0.1% in overall compositions observed *in situ* and on agar plates (summed).



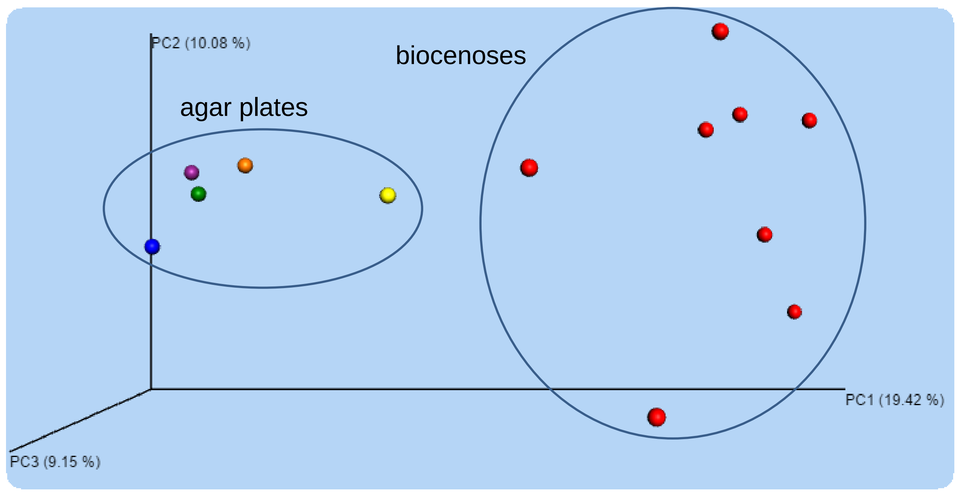




Fig. 5. Coordinates Analysis (Bray-Curtis) of differences between bacterial biodiversity observed *in situ* and on agar plates.

## Materials and methods

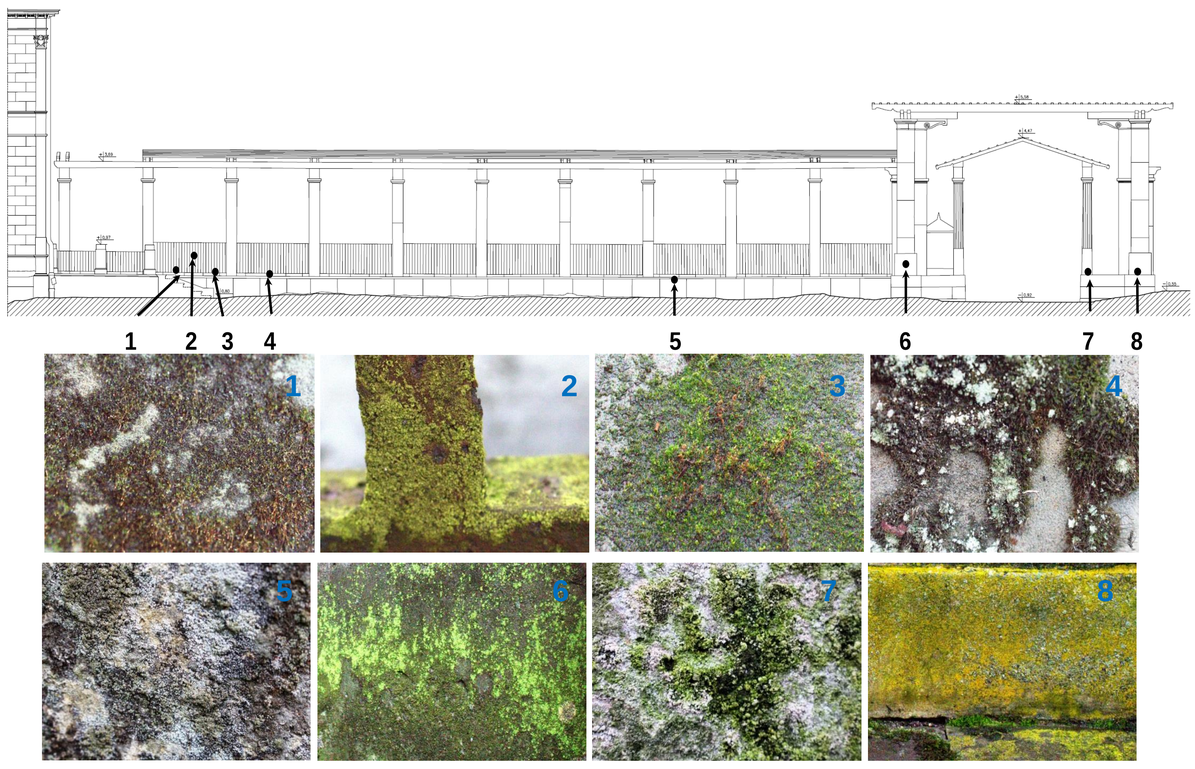



Fig. 1. Biocenosis photos of sampling sites on the Northern Pergola.

### *Sampling and microorganism cultivation*

The microbiological colonization of the Northern Pergola at the Museum of King John's III Palace at Wilanow (Warsaw; Poland) was assessed by the count plate method. Swabs were taken from different sampling sites on Pergola surfaces (described in Figure 1) from areas of 50 $cm^2$. The sterile sampling swabs were immersied in physiological solution (2ml) in the laboratory tubes, shaken, and spread (0.1ml) onto agar plates for bacteria or fungi: nutrient agar, Blickfeldt medium, Frazier medium, Czapek-Dox agar and Wort agar. The samples on nutrient agar were incubated at 37°C for 24h and other plates were incubated at room temperature (22–26°C) for 10 days.

### *Microorganism identification*

Samples of biocenoses with different morphologies were taken from the surfaces of the sandstone Pergola (Fig. 1). Total DNA was isolated and purified from the collected samples and from all colonies of microorganisms grown on various agar plates (Fig. 2). Total DNA isolation was performed using Power Soil® DNA Isolation Kit (Mo Bio Laboratories Inc). Subsequently, amplification and sequencing of the V3-V4 region of the 16S rRNA gene for *Bacteria* were performed for each sample. Illumina MiSeq technology was used for sequencing of each of the obtained amplicons.

Fig. 4. Bacterial biodiversity based on 16S bacterial rRNA gene analysis. Data for bacterial families constituted more than 1% in overall compositions observed *in situ* and on different agar plates. Two additional and artificial groups were created to include the families with abundance lower than the threshold (Other) and the ones with unspecified (i.e. '*f__*') or uncertain (i.e. '*Incertae_sedis*') taxonomic position assigned (Unclassified).

## Conclusions

- **The use of agar Wort plates enables the cultivation of bacteria in proportions and biodiversity most similar to these observed *in situ* in the analyzed biocenoses present on the historical sandstone surfaces.**
- **High-throughput sequencing methods allow for the determination of microbiological reference media most accurate for the specific applications described.**
- **Simultaneous application of the classical microbiology techniques and next-generation sequencing technology may enable reliable identification of environmental strains of bacteria, which, in the majority, are difficult to culture on the commonly used laboratory solid media.**

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