“Using long-read sequencing to explore the microbial community structures on salt-weathered historical buildings”

Monika Waldherr [1], Johannes Tichy [2], Guadalupe Piñar [2], Martin Ortbauer [3], Beate Sipek [3], Alexandra Graf [1]

[1] Department of Applied Life Sciences, University of Applied Sciences, FH Campus Wien, [2] Institute of Natural Sciences and Technology in the Art, Academy of Fine Arts Vienna,

[3] Institute for Conservation and Restoration, Academy of Fine Arts Vienna

Background

The continous degradation of historical buildings and artwork by different weathering processes is predicted to increase through lasting effects of climate change [1, 2]. To better understand the role of microorganisms in that procedure, we used long-read sequencing of 16S rRNA gene samples to identify the composition of the highly diverse microbial communities on different samples challenged by salt-weathering. This approach is independent of the culturability of the found microorganisms, hence it allows for a more comprehensive picture of the community composition [3]. Our results provide the basis for further characterizations and thereby contribute to the evaluation and development of more effective conservation methods in heritage science.

Analysis Workflow

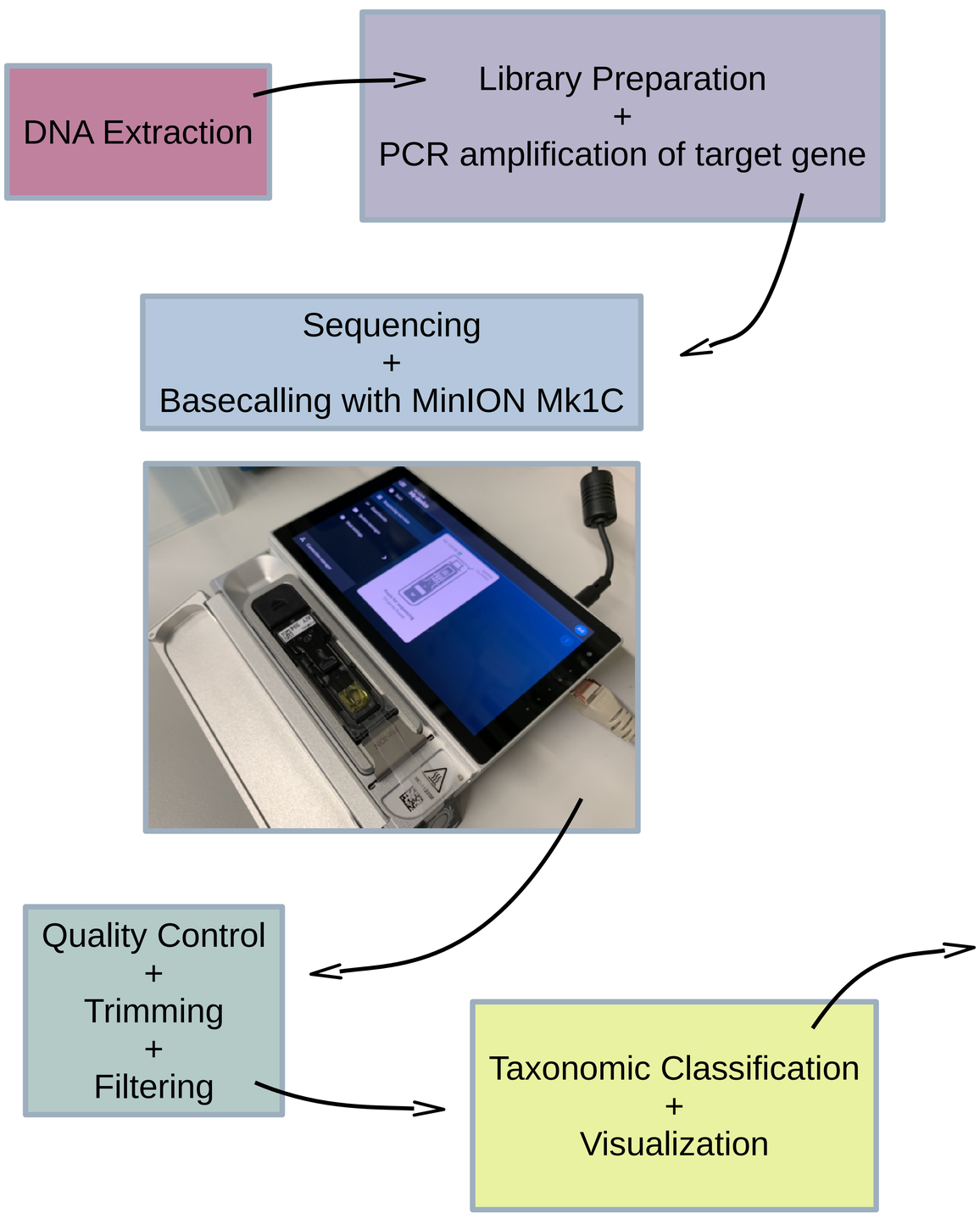

Figure 1: Schematic representation of the analysis workflow from DNA extraction of metagenomic samples to the final taxonomic classification results.

16S rRNA gene as Marker in Taxonomy

Different parts of ribosomal RNA (rRNA) gene sequences proved to be good markers for genetic barcoding. The reason for that is their ubiquitous presence in different organisms and their composition of variable and highly conserved regions. While the variable regions enable the discrimination of species, the conserved regions allow the binding of universal primers for targeted amplification. The 16S rRNA gene of Bacteria and Archaea is about 1500 basepairs long and includes nine hypervariable regions (V1-V9). An average gene sequence similarity of 98.65% is considered as threshold to discriminate between two species [4].

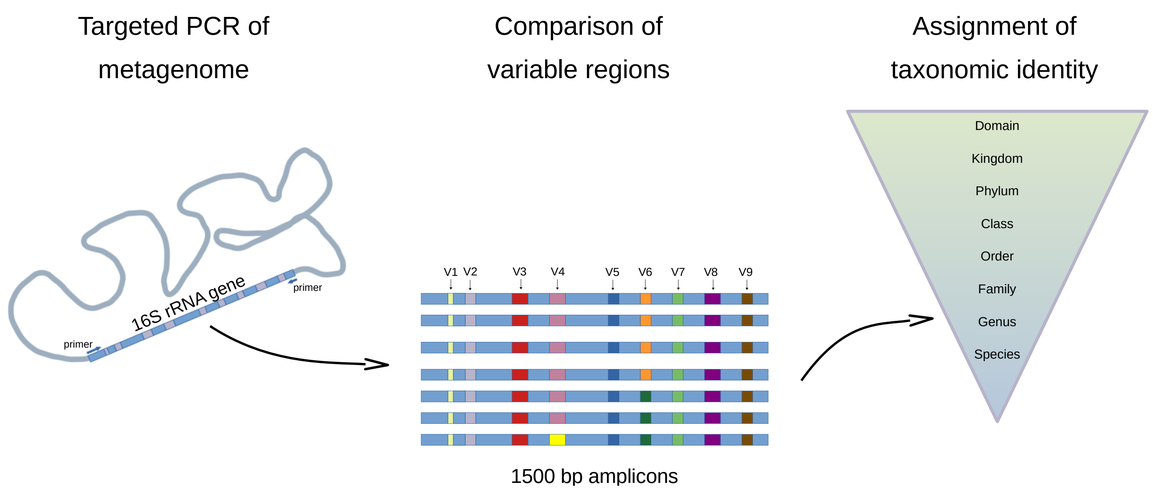

Figure 2: Visualization of the targeted 16S rRNA long-read sequencing of metagenomes to classify species belonging to the kingdoms of bacteria and archaea.

Results

Taxonomic classification results show an extremely high diversity of the studied metagenome. The highest abundance was detected for species belonging to the order of Rubrobacterales. Additionally, 30 other orders were identified. Only 4% of all reads could not be classified. Unfortunately, classification on species level is not possible without intensiv further bioinformatical processing. The reason for this might be the high error rate inherent to the nanopore sequencing technology in combination with additional errors introduced during PCR amplification. Nonetheless it is evident that the sample is dominated by Rubrobacterales with other interesting groups being present in low abundances.

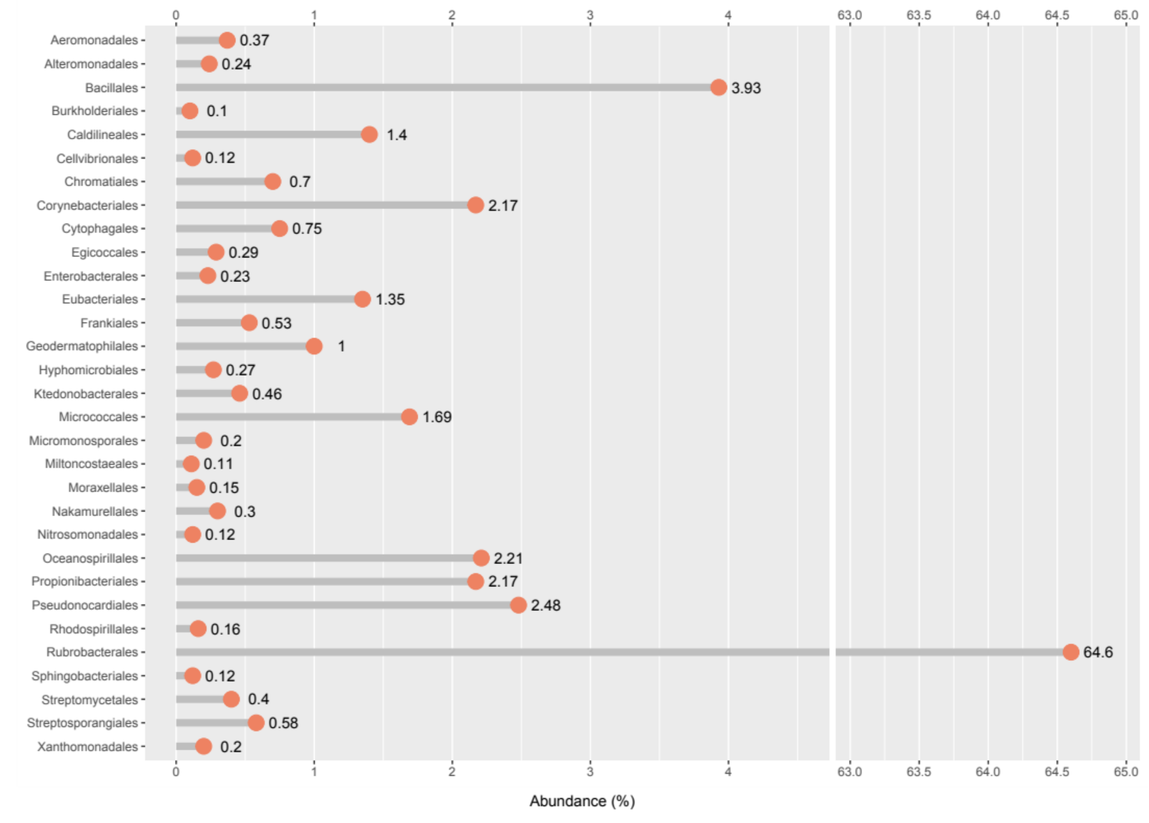

Figure 3: Microbial composition at order level of a metagenome sampled from a wall of the Charterhouse Mauerbach, Lower Austria. Only orders with an abundance higher than 0.1% are shown.

References

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Contact information:

monika.waldherr@fh-campuswien.ac.at,

alexandra.graf@fh-campuswien.ac.at