The microbiome of a XIV century medieval codex: are microbes part of cultural heritage objects?

Cecilia G. Flocco & Jörg Overmann
Department of Microbial Ecology and Diversity Research
Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH Inhoffenstrasse 7B, 38124 Braunschweig, Germany

Abstract: we present a comprehensive study of the microbiome of a medieval codex of the XIV century held at the Leipzig University Library (UBL), Germany. The overarching research hypothesis proposes microbes as integral parts of cultural heritage objects and potential biographical elements, challenging the current conservation practice narrative which labels microbes as detrimental to the objects. The selected object of study, Ms 12, is a parchment Bible which has not been digitized or made available for public use after its transfer from a nearby monastery to UBL facilities (presumably during the Reformation period), conditions that render it the appropriate object to study ancient microbial communities with minimal contemporaneous influence.

The experimental efforts focused on the development, optimization and application of a) sampling methods compatible with conservation requirements inherent to cultural heritage objects, b) targeted microbial cultivation techniques and c) next generation sequencing approaches, tailored to cultural heritage objects. We tested two sampling methods, swabbing and blotting, aiming at developing a robust, reproducible and minimally-invasive sampling approach suitable for application to different materials, shapes and locations within a codex and implementation in a library facility context, in the absence of dedicated microbiological equipment. An array of cultivation media, in some cases combined with enrichment techniques, was designed, aiming to retrieve a broad spectrum of microorganisms potentially present in the codex. Several folia were selected for sampling, making a distinction between homogeneous text passages and the margin areas that showed clear signs of handling (as evidenced by discolored and smudged sections). The microbial colonies obtained through cultivation were picked on regular intervals and purified for subsequent analyses and archiving purposes. Downstream analyses on purified colonies included microscopy (phase contrast and scanning electron microscopy), direct colony DNA extraction, followed by PCR amplification targeting a section of the 16s rRNA gene and sequencing with the Sanger method. Raw sequences were both automatically and manually curated and contrasted with data bases (SILVA, BLAST) to determine the percentual similarity values against described strains. Phylogenetic analysis allowed the identification of the isolates and the calculation of their similarity to known bacteria. While both sampling methods allowed the retrieval of numerous cultivable microorganisms, the swabbing method showed a better performance in terms of recovery of microbial entities and compatibility with downstream purposes. Approximately 400 microbial isolates were obtained, (including redundant ones) as a result of the cultivation effort. Endospore-forming microorganisms (*Bacillus spp.* and related microorganisms) were the predominant ones, followed by human and animal skin-associated microorganisms (*Staphylococcus spp*.), while no fungal entities were detected. Potential novel bacterial species were detected and selected strains are currently being described and identified through a polyphasic approach. Cultivation-independent microbiome analyses were run in parallel and the bioinformatic analysis is currently underway. A second manuscript, Ms 11, was recently discovered and the initial biographical

assessment indicates it could be a companion volume to Ms12. Ms 11 is currently subjected to the described microbiological survey, aiming to contribute microbiome information to help close gaps in the manuscript's biography, in an interdisciplinary approach.