

Nanopore sequencing and bioinformatics for rapidly identifying cultural heritage spoilage microorganisms

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Abstract

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Microbiological methodologies allow understanding the causes that lead to the development of a certain microbial community colonizing an artistic surface, to characterize its composition and describe its role in the deterioration of the constituent materials. Metagenomics allows identifying microbial communities directly in their natural environments, bypassing the need for isolation and cultivation of individual species, thus providing a more comprehensive picture of the biodiversity present on a surface compared with standard cultivation methods. Furthermore, molecular analyses require small amounts of material, favoring the preservation of the artistic surface during sampling. Here, we verified the suitability of a protocol consisting in DNA extraction with a micro-invasive sampling, using adhesive tape, PCR amplification with universal primers [Bacteria (16S), Fungi (ITS) and Viridiplantae (18S)] and amplicon sequencing by Oxford Nanopore technologies (ONT) in the hypogeum of the church of S. Nicola in Carcere "Basilica di San Nicola in Carcere" Church (Rome, Italy). Sequence data were analyzed with a bioinformatic pipeline customized in pinpointing cultural heritage spoiling organisms here named ALISIA. These data were integrated with traditional microbiology techniques that allowed the isolation of cultivable bacteria; three species were also characterized through their capability of biofilm formation and antibiotic resistance. Further, FTIR spectroscopy was performed to characterize the main products present on the masonry surface providing indications on the type of decay present.

This novel biological workflow represents a powerful opportunity to investigate the microbial colonization of artistic surfaces aimed at implementing preservation strategies of cultural heritage from bio-spoilage