

Assignment 1: Rapid Detection of Cultural Heritage Spoilage Microorganisms Using Nanopore Sequencing and Bioinformatics.

ABSTRACT AND INTRODUCTION:-

- Microbiological methodologies allow researchers to determine what causes a specific microbial population to colonise an artistic surface, as well as characterise its composition and role in the degradation of the constituent materials.
- Metagenomics allows identifying microbial communities directly in their natural habitats, bypassing the need for isolation and cultivation of individual organisms, resulting in a more detailed image of the biodiversity present on a surface.
- Molecular analyses only involve a small amount of material, allowing the artistic surface to be preserved during sampling.
- The sequence data was analysed using a bioinformatic pipeline called “AmpLlcon Sequencing Analysis,” which was created specifically for locating cultural heritage-degrading species (ALISIA).
- These findings were combined with conventional microbiology techniques to isolate cultivable bacteria; three species were also identified based on their biofilm formation and antibiotic resistance abilities.
- The key products present on the masonry surface were characterised using Fourier-transform infrared spectroscopy (FTIR) spectroscopy, which provided indications on the type of decay present.
- This novel biological workflow offers a unique opportunity to explore microbial colonisation of creative surfaces with the aim of developing bio-spoilage preservation strategies for cultural heritage.
- Cultural heritage (CH), such as sculptures, books, paintings, and frescoes, may be subjected to physical and chemical decay on a regular basis. Microorganisms can also interact with one another and with the environment, forming a true ecosystem with trophic chains and microbiological successions (Caneva and Salvadori, 1989).
- Biodeterioration can be exacerbated by biological contact with the CH (Sterflinger and Piar, 2013).

- Because of the emission of primary and secondary metabolism products and assimilation processes that can occur with or without the development of extracellular enzymes, microorganisms carry out micro- and macrosurface decontamination processes that cause physical damage and trigger chemical degradation processes (Pinna and Salvadori, 2005).
- The aesthetic damages are among the most noticeable, and they often jeopardise the work's readability. They are caused by mineral phase precipitation or the presence of biofilms, which may vary in thickness, microstructure, and colour.
- The colour of the biocenosis is determined by the stage of development and growth of the microorganisms involved, as well as the endo-pigments produced (Warscheid and Braams, 2000).
- The first significant and essential step in understanding the effects of microorganisms on CH is to adopt microbiological methodologies in order to understand the causes that lead to the formation of a specific microbial population, define its composition, and describe its function in the deterioration of the constituent materials.
- The presence of such microorganisms on the surface of a work of art does not imply that they are specifically responsible for its degradation. In reality, some microorganisms may arise as a result of secondary colonisation, and their behaviour is directed toward organic substances released into the environment as a result of pioneer species death and lysis (Radaelli et al., 2004).
- The only way to understand the position of each known species in the degradation process is to recreate the succession of populations on the surface (Caneva et al., 2008).
- Through a deeper understanding of the growth-limiting nutrients and the dynamics of biological aerosol diffusion for the conservation and preservation of monuments and artworks (González and Saiz-Jiménez, 2005; Caneva et al., 2008), microbiologists can recognise and improve strategies to control the growth of biodeteriogens and research appropriate prevention strategies (González and Saiz-Jiménez, 2005; Caneva et al., 2008).
- Microbial diversity can primarily be investigated using one of two methods: isolation and direct cultivation or indirect molecular analysis.
- Molecular methods are not bound by this restriction, allowing for a more complete and accurate image of the biodiversity present on the soil. Furthermore, by repeating molecular analysis at various points in time, valuable knowledge about the history of successive colonisation can be retrieved.
- Another benefit of using culturally insensitive molecular methods is the substantial reduction in the amount of material required for sampling. When

working on creative materials, this is a vital consideration to keep in mind in order to maintain their dignity.

- Metagenomics (Hugenholtz, 2002; Rappé and Giovannoni, 2003) is the application of modern genomics techniques to the analysis of populations of microbial organisms directly in their natural environments, bypassing the need for isolation and lab cultivation of individual species.
- Next-generation sequencing (NGS) metagenomics-based techniques represent a minimally invasive and effective opportunity to investigate the microbial communities, structure, and functional diversity that may be present on artistic surfaces or in various environments ranging from soil to the human gut (Gutarowska et al., 2015).
- Cultural-dependent approaches should ideally be used in conjunction with molecular analysis investigation methods to allow for the study of the physiology and metabolism of isolated microorganisms as well as an understanding of their possible function in biodeterioration (Qi-Wang et al., 2011; Otlewska et al., 2014).
- The aim of this study is to determine the microbiological composition of the hypogeum of one of the most iconic Roman monuments for architectural and historical formation: the Basilica di San Nicola in Carcere Church in Rome, using molecular methods such as ONT sequencing. Three temples are included in the structure, as well as a mediaeval tower that has been converted into a bell tower.
- The Church shares the basement and part of the structures with three Roman temples below; its location near the Tiber River has made it vulnerable since its first construction in 1128 AD. Indeed, the high level of humidity present in the masonries made necessary the periodic reconstruction of its frescoes, which in the last version date back to the XIX century.
- We conducted micro-invasive hypogeum sampling with adhesive tape; due to low material recovery, we added a PCR amplification stage of the total DNA collected, using universal primers for three kingdoms, 16S for bacteria, ITS for fungi, and 18S for Viridiplantae.
- The amplicons were then sequenced by ONT using a metagenomics approach, and the output data was analysed using a tailored bioinformatic pipeline. Microbial and chemical characterizations were carried out in parallel from the same sampling area.
- To emphasise the importance of a combined approach to investigate biodegradation phenomena, cultivable bacteria were evaluated, and three isolates were analysed in terms of antibiotic resistance and biofilm formation capacity.