

Assignment 2: Rapid Detection of Cultural Heritage Spoilage Microorganisms Using Nanopore Sequencing and Bioinformatics.

Related work :-

- Dealing with CH, the primary concern is that of the preservation of the artistic surface. In this context, sampling must be performed adopting the least invasive method possible and collecting the smallest amount of material required for analysis. The micro-invasive sampling through adhesive tape only causes minimal damage to the superficial layer of the artistic object.
- Microbiological components that may have colonised the porous cavities are omitted since this sampling method only allows sampling of the surface of the artistic piece. This sampling procedure, it was discovered, allows for the extraction of sufficient amounts of DNA for genomic analysis without the need for prior amplification.
- To get around this issue, we can use PCR amplification of rDNA kingdom-specific regions to exponentially amplify DNA to make it suitable for NGS analysis. The ONT MinION sequencer helps you to easily sequence samples directly in your laboratory for real-time analysis. More traditional sequencing technologies necessitate a longer period of waiting for sequencing results.
- The large number of reads linked to amplicons from various species sampled by micro-tape in the hypogeum of Basilica di San Nicola in Carcere Church were decrypted using ALISIA, a bioinformatic tool specifically designed for this purpose.
- ALISIA is a useful bioinformatics platform that allows you to quickly analyse raw amplicon sequencing data from a variety of sequencing technologies, including Illumina and ONT. ALISIA offers a direct and immediate view of the microbiome present on the examined CH when used in CH metagenomics studies.
- Many organisms were included in the microbiome obtained by the adhesive tape sampling system in the three kingdoms, according to ALISIA. BLAST research attributed the most abundant reads to uncultured microorganisms, despite the vast number of species present in both the fungi and bacteria kingdoms.

- This is due to the impossibilities and difficulties that conventional cultural methodologies have in isolating microorganisms (Amann et al., 1995; Hanson et al., 2016). As a result, obtaining confirmation of the taxonomic results' reliability proved difficult.
- ALISIA also noted the existence of several species of the genus *Nesterenkonia*. The Micrococcaceae family contains mesophilic mild haloalkaliphilic species in this genus (Stackebrandt et al., 1995). *Kocuria*, a closely related genus (Stackebrandt et al., 1995), was isolated and described by molecular analysis even though this genus was not present on the bacteria isolation.
- It's difficult to compare metagenomic analyses' high precision to the isolation and cultivation of individual organisms. A functional metagenomics analysis can be used to identify the degradation molecular processes involved in the analysed CH, bypassing the identification stage entirely and acting directly on the molecular pathway.
- Despite the fact that this was a bacteria-focused study, a large number of fungal species were discovered, all of which are likely involved in CH deterioration. Nonetheless, findings in 18S amplicon sequencing indicate an advantage in ONT and ALISIA's accuracy: the majority of the plant species identified are tree plants popular in Rome, such as *Platanus*, *Pinus*, or *Cupressus*, or herbaceous plants typical of cracks in rock faces: *P. judaica* is a *judaica* species. Pollen accumulated on sampled wall surfaces most likely contains the DNA of these plants. ONT MinION's metagenomic amplicon sequencing reveals itself to be a fast method for investigating the taxa present on the surface of CH.
- Cultivable bacteria were isolated to learn more about the bacterial composition of the hypogeum's masonry. Although several of them, such as *Bacillus*, *Kocuria*, *Paenibacillus*, and *Arthrobacter*, were found to be involved in the development of salt efflorescences (Laiz et al., 2000), *Pseudomonas* spp. were found to be involved in the production of calcite (Rivera et al., 2018).
- Three strains from the Gram-positive *P. pabuli*, *K. rhizophila*, and *B. licheniformis* species, and one from the Gram-negative *S. maltophilia* species, were chosen as members to be further characterised. As several studies have shown, they are all interested in biodeterioration (Tarsitani et al., 2014; Saiz-Jimenez, 2017).
- *P. pabuli*, in addition to microbial glass corrosion, is implicated in the biodeterioration of historic limestone buildings (Marvasi et al., 2009; Skipper and Skipper, 2016); *B. licheniformis*, on the other hand, is a facultative anaerobe that has been found on damaged paintings (Marvasi et al., 2009; Skipper and Skipper, 2016). (Capodicasa et al., 2010).

- *Bacillus*, *Kocuria*, and *Paenibacillus* spp. have been found to develop in the presence of nitrate on organic substrates without oxygen and have been linked to salt efflorescence (Laiz et al., 2000).
- Nitrifying bacteria convert ammonia to nitrite and nitrate ions, which result in the formation of nitric acid. This process results in stone dissolution and the formation of soluble nitrate salts, which appear as efflorescence; humidity also plays a role in this process (Kumar and Kumar, 1999).
- The Basilica di San Nicola in Carcere Church, which is located near the Tiber River, has a high humidity level, which encourages microorganism development. Humidity can react with stone matrixes, causing deterioration and, in particular, dissolution of carbonates, solubilization of other elements, and, as a result, material erosion.
- Microorganisms, in combination with abiotic influences, use stone surfaces as a base, causing erosion and aesthetic harm (Sterflinger and Piar, 2013; Dubey and Jain, 2014).
- The processing of inorganic acids like nitrous and nitric acid, as well as salts like nitrite and nitrate, by nitrifying bacteria is known to cause biodeterioration (Mansch and Bock, 1998).
- The existence of nitrate residues and gypsum on salt efflorescences was revealed by FTIR research, which strongly correlated with the metabolism of *Bacillus*, *Kocuria*, and *Paenibacillus* species isolated from the hypogeum wall (Laiz et al., 2000; Castrillón Rivera et al., 2019). (See Figure.)
- In agreement, soluble calcium carbonate is converted into calcium sulphate, i.e. gypsum, at high humidity, resulting in clear salt efflorescence (Atlas et al., 1988).
- *K. rhizophila* IP5, *B. licheniformis* IP7, and *S. maltophilia* IP9 were also able to form biofilms among the isolated bacteria. Since microorganisms can withstand harsh abiotic conditions and many biocides, as well as deteriorate manufactured goods, bacterial aggregation is the primary cause of biodeterioration (Stewart, 2002; Dakal and Cameotra, 2012; Rivera et al., 2018).
- This was verified by an antibiotic susceptibility test, which showed several resistances for various strains; indeed, certain resistance mechanisms have been stated to be common to both biocides and antibiotics (Scientific Committee on Emerging and Newly Identified Health Risks [SCENIHR], 2009).
- In recent years, new technologies have been developed to tackle biodeterioration and restore stone structures using bacterial metabolism to solve problems related to biocide resistance.
- The eco-friendly, self-healing, and highly robust design of microbially induced carbonate precipitation has been investigated as a promising technology for the bioremediation of such structures.