Assignment 3:Rapid Detection of Cultural Heritage Spoilage Microorganisms Using Nanopore Sequencing and Bioinformatics.

ABSTRACT AND INTRODUCTION:-

- Microbiological methodologies allow researchers to determine what causes 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 "AmpLIcon 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).

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 MiniION 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.

Results and Methodology:-

Bacteria Isolation and Sampling for Molecular Analysis

- To study the existence of microorganisms on CH surfaces, two alternative techniques were used: separation of microorganisms for nanopore sequencing analysis and isolation of cultivable bacteria for Fourier-transform infrared spectroscopy (FTIR) analysis. Biological material was collected for sequencing analysis utilising a non-invasive or micro-invasive collection procedure for CH and adhesive tape (Urz and De Leo, 2001).
- This non-invasive sample methodology enables for the collection of sufficient biological material without jeopardising the CH's integrity. The sticky tape sampling approach was employed to gather samples for metagenomics

analysis along the hypogeum wall of the Basilica di San Nicola in Carcere Church in Rome.

- The samples collected (n = 6) were combined into two samples (Samples 1 and 2) and kept at 20°C until the DNA extraction processes. Cotton swabs were used for sampling extremely close to the area where adhesive tape sampling was done in the situations of cultivable bacteria isolation and FTIR analysis.
- Cotton swabs were firmly pressed on the wall's surface and then inserted in sterile tubes containing 2 mL of Nutrient Broth (NB). Samples were cultured at 30°C for 24 hours in the laboratory before being plated on NB agar plates. Isolation and purification of morphologically distinct colonies was possible using plates incubated at 30°C for 24 hours. The amplification of 16S rDNA was used to identify bacterial isolates at the molecular level.
- Using specific rDNA 16S, ITS, and 18S region primers, PCR amplifications were performed on the two sampling pools (Samples 1 and 2) derived from the hypogeum of Basilica di San Nicola in Carcere Church (see section “Materials and Methods”) to investigate the presence of bacteria, fungi, and Viridiplantae (see section “Materials and Methods”).
- For all three regions studied, electrophoresis agarose gels confirmed the presence and length of amplification products. With each kingdom-specific primer, both genomic DNA from Sample 1 and Sample 2 yielded amplicons: I ITS (fungi), one band at 600 bp in Sample 1 and two separate bands at 600 and 900 bp in Sample 2; (ii) 18S (Viridiplantae), both samples yielded a single band at 1,800 bp; and (iii) 16S (bacteria), single band at 1,500 bp in both samples (Figure 1A).
- A light smear could be seen near the expected size bands in every PCR run, which could indicate the presence of DNA from several microorganisms. ONT MinION sequencing was used to quickly classify the PCR amplicon products: for Samples 1 and 2, the run generated 6 Mb of data (100 k reads) and 11 Mb of data (190 k reads), respectively.
- In order to pinpoint CH spoiling organisms, a customised bioinformatic pipeline called ALISIA was used. The sequenced reads were analysed using the four basic steps outlined in the “Materials and Methods” section. In the first two steps, a taxon was assigned to 94,353 reads in Sample 1 and 155,506 reads in Sample 2 (80 percent of the total reads).
- In the third step, 21,597 reads for Sample 1 were assigned to bacteria, 47,729 to fungi, and 5,571 to Viridiplantae, while 34,803 reads for Sample 2 were assigned to bacteria, 36,176 to fungi, and 1,055 to Viridiplantae (Figure 2). However, 19,456 reads for Sample 1 and 83,472 reads for Sample 2 were assigned to the Metazoa and Protista kingdoms.