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http://www2.warwick.ac.uk/fac/sci/lifesci/research/jcmurrell/currentprojects/culturalheritage/

Cultural Heritage (CH) outdoor stoneworks can be damaged by physical, chemical and biological processes caused by microorganisms. To effectively protect and maintain long-term conservation of European CH stonework, it is vital to identify the active components of the microbial community that cause damage in the biodeterioration processes and define their specific roles.
This project focused on the identification of the active "key players" in the biodeterioration processes and on understanding their function and activity using advanced techniques in microbial molecular ecology.
The project had a multidisciplinary approach, based on microbiology, molecular biology, and biogeochemistry essential for a better understanding of the microbial diversity and functional activity in the processes that alter our CH patrimony. The project comprised:

1. An ***in situ* study** on altered CH stone samples and identification of the active key players of the microbial community (University of Warwick, Murrell and Purdy Laboratories)
2. **Laboratory chamber studies** on artificially enriched stone discs to monitor development and growth of the microbial community using accelerated weathering chambers (University of Portsmouth – May and Inkpen Laboratories, and University of Warwick, Murrell and Purdy Laboratories).

Sandstone alterations (exfoliation and black crust) at a key study site, Portchester Castle near Portsmouth, were considered; we aknowledge and thank English Heritage for giving us the permission to sample this historic site.

Objective 1. A better understanding of the structure and the function of the microbial community colonising and altering CH stonework by using advanced molecular techniques

Methods: DNA and RNA extractions from altered CH stone samples. DNA- and RNA-Denaturing Gradient Gel Electrophoresis analyses, Reverse Transcriptase-PCR, Functional gene analysis, Restriction Fragment Length Polymorphism, 16S rRNA gene sequence analysis, 454 pyrosequencing.
Outcomes: In stone alterations, a higher diversity in the Bacterial community compared to the Archaeal community was observed. Qualitative and quantitative differences in the prokaryotic community were revealed between the microbial communities present in the exfoliations and those in the black crust. The presence of microorganisms well adapted to "extreme" environments was observed, especially regarding the ability to tolerate high salt concentration, arid, high UV radiation and oligotrophic condition. The most predominant microbes identified included Chloroflexi, Actinobacteria, Deinococcus, α- and β- Proteobacteria, Cyanobacteria, Bacteroidetes and halophilic Archaea. An important outcome was the use in these CH biodeterioration studies of next generation sequencing data analysis (454 pyrosequencing). Functional gene analysis revealed the presence of both Archaeal and Bacterial ammonia oxidizers- (*amoA* genes) and denitrifying bacteria (*nirK* genes). In particular bacterial ammonia oxidizers were related to the genus *Nitrosospira* also previously found on stone surfaces.

Objective 2. Detection of metabolically active microorganisms present in samples and isolation studies of the "key players" and more active strains in the biodeterioration process

Methods: cultivation studies, 16S rRNA gene sequence analysis, 454 pyrosequencing of functional and 16S rRNA genes.
Outcome: 454 pyrosequencing analyses revealed the presence of metabolically active key players including *Rubrobacter* and *Sphingomonas* and *Halococcus*, respectively for the bacterial and archaeal community.
An important outcome was also the evidence in the exfoliated samples of bacterial *amoA* gene expression, confirming that detected ammonia-oxidisers bacteria, including *Nitrosospira*, *Nitrosomonas* and *Nitrosovibrio* are active on stone.
Isolation studies revealed the presence of *Bacillus* and *Arthrobacter* and potentially halotolerant *Streptomyces*, *Micrococcus* and *Pseudomonas* confirming the importance of salt adaptation in the altered stone samples.

Objective 3. Information on the microbial succession in the surface colonisation of stone discs under controlled lab conditions using weathering chambers

Methods: DNA and RNA extractions from stone discs, 454 pyrosequencing, Electron Microscopy and EDX analyses.

Outcome: 454 pyrosequencing analyses facilitated observations of microbial community succession over 5 months. This revealed the adaptability to grow on stone of some bacterial species including *Pseudomonas*, *Stenotrophomonas*, *Bacillus*, *Arthrobacter* and *Paenibacillus*. Electron Microscopy and EDX analyses were excellent tools to observe stone surface colonisation, structural analysis and damage assessment in both the altered samples from Portchester Castle and the colonised stone discs.

In conclusions, the project allowed us to determine the role and activity of specific groups of microorganisms in CH altered samples, to improve knowledge of the analysis of the structural composition and diversity of the microbial communities and to acquire a comprehensive understanding of microbial succession in the colonisation processes used in our experiments. The approaches used in this study can be applied in future studies in the CH field. In particular, it will be important for scientific conservationists to understand the microbial populations and activities of microbes on stone artefacts in order to formulate preservation strategies and prevent further biodeterioration. The outcomes of this research are being prepared for publication in both specialist microbial ecology journals and cultural heritage/biodeterioration-specific journals.

The website created for dissemination of research results to a scientific and non-scientific audience reports on the research program and conveys the importance and interest of the work in the context of CH; thereby promoting public understanding of science. Dr Zanardini was also involved in the supervision of an undergraduate project student at Warwick who worked for 10 weeks on aspects of this project, thereby exposing younger scientists to the field.

During the project, the Marie Curie Fellow, Dr Zanardini, considerably improved her research skills both in terms of key molecular biological techniques for the study of structure and function of the microbial community and also through the interpretation of DNA and RNA datasets (bioinformatics).

Socio-economic impact

The European Union plays a central role in preserving the heritage of its member and affiliated countries. There is a social and political need to maintain awareness of cultural diversity within Europe and to conserve cultures and traditions which are also embodied in stone materials. The most obvious benefit of the preservation and conservation of objects of art and historical sites is an increase in tourism, leading to an increase in economic activity and education. The major benefits derived from the project are: a) new collaborations and a network between Universities, Organizations, Research Institutions in Europe have been developed and improved giving excellent opportunities for future collaborative research projects with a European dimension; b) creation and development of European research teams which have the potential to reach a high level of excellence and so Europe can become the world-leader in research into the microbial communities involved in the biodeterioration of CH; c) a trans-national and multidisciplinary team (Universities of Warwick, Portsmouth, Insubria, Milan, Molise, Glasgow, Newcastle, Cambridge, Rome, Centre for Ecology & Hydrology, Oxford, Research & Testing Laboratory, Lubbock, Texas, USA) to investigate fundamental issues in microbial ecology applied to the biodeterioration of outdoor CH stoneworks; d) awareness that new techniques can be applied to CH studies and that a highly trained work force can be developed in Europe which will can then be in an excellent position to tackle these CH issues in the future.

The biggest contribution to European Excellence and Competitiveness is also a new dimension into the microbial studies on biodeterioration of outdoor CH artefacts through extensive training in a number of new, cutting edge technologies in environmental microbiology which can be directly applied to CH problems i.e. what microbes are present, what are they doing and how they can be prevented from "spoiling" historical works of art or indeed how they can be also exploited to preserve or clean CH artefacts (biorestoration).

Impact for the Marie Curie Fellow

The benefits to the career of Marie Curie Fellow Dr Zanardini are that she significantly developed her practical research capabilities and complementary skills and had the opportunity to contribute to several research projects. New collaborations were developed using interdisciplinary approaches. Experience of international collaboration and research carried out abroad are considered an essential requirement for an improved scientific career. The experience of international collaborations and research has maken major contribution to Dr Zanardini's professional performance and will accelerate her career development, bringing a significance advance in her practical research capabilities and complementary skills far beyond the lifetime of the project. In addition, Murrell at the host laboratory has been stimulated by a new field of research and will continue to collaborate with Dr Zanardini and the CH network that has been created during the project.