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Characterization of aerobic bacterial and fungal microbiota on surfaces of historic Scottish monuments ☆

Maija-Liisa Suihko[a,*], Hanna-Leena Alakomi[a], Anna Gorbushina[b], Irene Fortune[c], Jürgen Marquardt[b], Maria Saarela[a]

[a] *VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Espoo, Finland*
[b] *Institute for Chemistry and Biology of the Marine Environment (ICBM) Geomicrobiology, Material Ecology, Carl-von-Ossietzky-Str. 9-11, 26111 Oldenburg, Germany*
[c] *Historic Scotland, 7 South Gyle Crescent, Edinburgh EH12 9EB, Scotland, UK*

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Abstract

Twenty samples were taken from the inner or outer surfaces of stone monuments of six historic Scottish buildings and ruins. Biofilms developing on mineral substrates were analysed by *in situ* scanning electron microscopy and cultivation. Various methods were used to characterize the isolates including automated ribotyping, RAPD and sequencing of the 16S rRNA gene for bacteria, and stereomicroscopy and sequencing of the Internal Transcribed Spacers (ITS) for fungi. Most samples contained microbes between 10^5 and 10^7 cfu g^{-1} substrate. Actinobacteria belonging to the genus *Streptomyces* (17 samples/5 monuments) or *Arthrobacter* (12/3) and *Pseudomonas* (9/3) were frequently detected. Most streptomycetes were in terms of their 16S rRNA gene sequence most closely related to *S. microflavus* (10/3) or to the undescribed species *S. "vulgaris"* (8/3). Indoor and outdoor biofilms exhibited significant differences in their microbiota, as shown by both microscopy and isolation studies. Pigmented coccoid *Arthrobacter* species were typical for the outdoor samples, whereas *Pseudomonas* species were common in the indoor samples. Based on the low phylogenetic relationship to a known species (type strain), potential novel pigmented bacterial species belonging to the genera *Arthrobacter*, *Brevundimonas*, *Cryseobacterium*, *Deinococcus* and *Dyadobacter* were detected from the outdoor samples and to *Pseudomonas* from the indoor samples. Hyaline fungal species of *Acremonium* (10/4) mainly occurred in indoor samples, whereas pigmented species of *Cladosporium* (8/3), *Penicillium* (6/3) and *Phialophora* (6/2) were found outdoors. Using *in situ* microscopy diatom algae were also detected.

Keywords: Historic monuments; Biofilms; Actinobacteria; *Streptomyces*; *Arthrobacter*; Fungi; *Cladosporium*; *Phialophora*; Protective pigmentation

Introduction

During recent decades there has been a general concern about the deterioration of historic buildings. Along with chemical and physical weathering factors, microbial growth plays an important role in this process

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*Corresponding author. Tel.: +358 20 722 5133; fax: +358 20 722 7071.
E-mail address: Maija-Liisa.Suihko@vtt.fi (M.L. Suihko).

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[7,10,14,21,36,38,54]. Stone type and local climatic differences, e.g. exposure to light and humidity, and the supply of nutrients for the growth of microbes, have a great impact on the biodeterioration processes and their outcomes. Microbial metabolism results in deteriorating agents such as organic and inorganic acids, chelating agents, enzymes and extracellular polymeric substances (EPS), causing e.g. biocorrosion and biomineralization [5,54]. Furthermore, phototrophic and some heterotrophic filamentous microbes (streptomycetes and fungi) are able to penetrate into stone materials [9,49,54]. In addition to structural damage, rock biofilms also cause aesthetic damage, since several microbes produce pigments as a protection against UV-radiation, chemicals and other environmental stress factors [8,11,19,39].

Surfaces of old stone buildings may be colonized by algae, cyanobacteria, lichens, mosses, chemolithotrophic bacteria, heterotrophic bacteria and fungi, protozoa and a variety of small animals [20,21,54]. Biofilm formation on clean surfaces of new buildings usually starts with phototrophic organisms (algae, cyanobacteria), which use CO_2 from the atmosphere as their carbon source and sunlight as their energy source. Chemolithotrophic bacteria (e.g. thiobacilli, nitrifying bacteria) obtain energy by oxidation of inorganic compounds and fix CO_2 from the atmosphere [5]. Heterotrophic organisms (most bacteria and all fungi) need some organic carbon source for their growth, which is provided by metabolites of phototrophic organisms or by air-borne deposition. It has been shown that the very low nutrient requirements of some rock-inhabiting heterotrophic organisms (actinobacteria, black fungi) may be fulfilled by organics of polluted air and rain [38,53] or of animal remains and excretions [20].

Microbiota on monument surfaces vary greatly in different geographical zones, as well as with changes of atmospheric and microclimatic conditions. Biocides used to prevent the growth of microbes are toxic chemicals, which may also affect higher organisms including humans [14] and should be used only in exceptional circumstances. Often, risks associated with repetitive use of common biocides are deemed to be greater than not treating the surface. Site-specific rock biofilm microbiota has to be considered in all conservational practices for cleaning and prevention of recolonization of monument surfaces. In order to develop ecological and effective biocontrol procedures, detailed characterization of the biofilm communities of the monuments is necessary. In this respect, the aerobic cultivable (active) microbiota, especially microbes able to start the colonization, has a central role.

Culture-independent methods, such as DGGE, TGGE and SSCP, have been proposed to result in a better general overview of the dominant *in situ* population, including uncultivated and inactive (dead) microbes, than culture-based methods [16,23,40]. However, cultivation of microbes from biofilms enables more practical characterization of their deteriorative potential and generates isolates which can be characterized in more detail by different molecular biological methods and can be used for development of new biocontrol methods.

This work was a part of EU-project EVK4-CT-2002-00098 ''Inhibitors of biofilm damage on mineral materials''. The aim of the study was to characterize the aerobic microbiota on the surfaces of Scottish stone monuments, with special attention to bacterial and fungal species which could start the colonization, as well as to provide relevant strains for further studies. In addition, the biofilms were analysed by *in situ* scanning electron microscopy.

Materials and methods

Climate conditions

The historic monuments examined in this study are in the care of Historic Scotland and are generally located on the eastern side of the country. Scotland has a temperate, humid climate with rainfall levels between 0.8 and 3 m/year. Sunshine hours on the east coast may reach 1400 h/year. Annual daily average temperatures are 7–9 °C in lowland areas (UK Meterological Office) and decrease with increasing height above sea level. The coldest months are January and February, when average temperatures in lowland areas are 5–7 °C. In coastal regions, winter temperatures are dependant on sea surface temperatures and are generally far milder than in inland areas (UK Meterological Office). The warmest months are July and August, with daily maximum temperatures in lowland areas of approximately 19 °C. In addition, eastern coastal areas experience sea fogs between April and September. The monuments included in the study vary in their location (urban, rural and coastal) and each site is subject to particular microclimates dependent on orientation of the building and the presence of adjacent buildings or trees. Furthermore, location influences the type and amount of atmospheric compounds deposited on external surfaces (e.g. salts, combustion particles and organic aerosols), with implications for the diversity of microbes present.

Sampling and isolation

Samples were taken in June 2004 from six different Scottish historic monuments showing evidence of biological colonization. Detailed descriptions of the sampling places and stone materials are presented in Table 1. The samples were classified as indoor or