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

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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.
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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

☆The GeneBank accession numbers of streptomycetes are EF093107–EF093122 and EF564804–EF564808. The numbers of other bacteria are EF093123–EF093135 and DQ465009–DQ465010. The numbers of fungal sequences are AM410602–AM410612.

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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

Table 1. Description of sampling places

Monument	Sample	Site	Biofilm colour	Material
Arbroath Abbey ruins (dating from 1178)	Abbey 1	Outer surface, no roof (coastal, exposed)	Greenish	Red-brown sandstone, medium grained. Probably from the lower devonian period, porous and showing decay
	Abbey 2	Outer surface, no roof (coastal,exposed)	Greenish	
	Abbey 3	Outer surface, no roof (coastal, exposed)	Green-brownish	
	Abbey 4	Outer surface, no roof (coastal, exposed)	Greenish	
Linlithgow Palace ruins (built 1424–1621)	A1	Inner wall, no roof (inland, semi-rural)	Greenish	Cream-orange sandstone, thinly bedded and from the Carboniferous period
	A2	Inner wall, no roof (inland, semi-rural)	Greenish-brownish	
	A3	Inner wall, no roof (inland, semi-rural)	Greenish-brownish	
	A4	Inner wall, no roof (inland, semi-rural)	Greenish-brownish	
	A5	Inner wall, no roof (inland, semi-rural)	Greenish-brownish	
St. Andrews Castle (dating from 1400)	STA1	Underground tunnel, artificial light (coastal, exposed)	Greyish	Grey calciferous sandstone, bedrock from the Carboniferous period
St. Serf's Church (1150 and later rebuilding)	S6	Inner wall (inland, rural)	Black	Plaster
Stirling Castle (existing site from 1501)	S1	Inner wall (inland, urban)	Green-brownish	Sandstone painted with lime wash
	S2	Inner wall (inland, urban)	Green-brownish	
	S3	Inner wall (inland, urban)	Green-brownish	
	S4	Inner wall (inland, urban)	Greenish	
	S5	Inner wall (inland, urban)	Brownish	
Tolquhon Castle ruins (dating from 1420)	T1	Inner wall, no roof (inland, rural)	Greenish	Pink metamorphosed sandstone, (psammite) dominated with quartz and containing feldspar and calcium silicate bands
	T2	Inner wall, no roof (inland, rural)	Black-grey	
	T3	Inner wall, no roof (inland, rural)	Greenish	
	T4	Inner wall, no roof (inland, rural)	Green-brownish	

outdoor samples depending on the presence of a roof. Altogether 13 outdoor (3 monuments) and 7 indoor (3 monuments) samples were analysed (Table 2). The microbial groups examined were actinobacteria, determined on actinomycetes isolation agar (AIA, Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 0.05% cycloheximide at 25 °C for 21 d, other aerobic heterotrophic bacteria determined on R2A medium (Difco) at 25 °C for 14 d and fungi determined on potato dextrose agar (PDA, Difco) supplemented with 0.01% chloramphenicol, 0.01% chlortetracycline and 0.02% Tween Triton x-100 at 25 °C for 14 d. At two sampling sites, swab samples (S6, STA1) were taken with sterile cotton swabs. At the other sites, surface biofilms with some substrate (1–2 mm) was carefully scraped with a scalpel. The microbial counts in samples (cfu) were obtained from dilution series. The detection limit was 1000 cfu g^{-1}. From each sample at least two

Table 2. Bacterial and fungal microbiota of different samples

Monument sample	Bacteria on AIA agar ($n = 79$)		Bacteria on R2A agar ($n = 59$)		Fungi ($n = 76$)	
	cfu g^{-1}	Closest species (no of isolates)	cfu g^{-1}	Closest species (no of isolates)	cfu g^{-1}	Closest species (no of isolates)
Arbroath Abbey (outdoor)						
Abbey 1	10^6	*Streptomyces candidus* (1) *Streptomyces cirratus/avidinii* (1) *Streptomyces microflavus* (1)	10^7	*Arthrobacter agilis* (2) *Arthrobacter tumbae* (1) *Chryseobacterium taiwanense* (1) *Pseudomonas* sp. (3)	10^5	*Cladosporium* sp. (2) *Penicillium* sp. (2) *Phialophora lignicola* (2) *Torrubiella confragosa* (2)
Abbey 2	10^5	*Streptomyces microflavus* (2)	10^6	*Arthrobacter agilis* (2) *Arthrobacter tumbae* (1) *Pseudomonas mandellii* (2)	10^6	*Cladosporium* sp. (2) *Penicillium* sp. (2) *Phialophora lignicola* (2) *Trichothecium domesticum* (2)
Abbey 3	10^6	*Streptomyces "vulgaris"* (3)	10^7	*Arthrobacter agilis* (2) *Deinococcus marmoris* (1) *Pseudomonas mandelii* (2)	10^7	*Cladosporium* sp. (2) *Phialophora lignicola* (2) *Torrubiella confragosa* (2) *Trichothecium domesticum* (1)
Abbey 4	10^6	*Streptomyces "vulgaris"* (7)	10^6	*Arthrobacter agilis* (2) *Arthrobacter parietes* (1) *Deinococcus marmoris* (1)	10^7	*Penicillium* sp. (2) *Phialophora lignicola* (2) *Torrubiella confragosa* (2)
Linlithgow Palace (outdoor)						
A1	10^6	*Streptomyces microflavus* (1) *Streptomyces atroolivaciens/olivoviridus* (1)	10^4	*Arthrobacter parietes* (2) *Arthrobacter roseus* (2)	10^4	*Acremonium* sp. (1) *Penicillium* sp. (2) *Phialophora lignicola* (2)
A2	10^5	*Streptomyces microflavus* (2)	10^4	*Arthrobacter tumbae* (2)	10^5	*Acremonium* sp. (2) *Cladosporium* sp. (2) *Phialophora lignicola* (2)
A3	10^5	*Streptomyces microflavus* (14) *Streptomyces "vulgaris"* (4)	10^5	*Arthrobacter tumbae* (2)	10^4	*Cladosporium* sp. (2)
A4	10^6	*Streptomyces atroolivaciens/olivoviridus* (4)	 10^5	*Arthrobacter parietes* (1) *Arthrobacter roseus* (1)	10^3	*Cladosporium* sp. (1) *Penicillium* sp. (2)
A5	10^6	*Streptomyces microflavus* (2) *Streptomyces "vulgaris"* (1)	10^6	*Arthrobacter parietes* (1) *Arthrobacter roseus* (1)	10^3	*Acremonium* sp. (2) *Cladosporium* sp.(2) *Fusarium* sp. (1)
St. Andrews Castle (indoor)						
STA1	10^4	*Streptomyces avidinii* (1) *Streptomyces spiroverticillatus* (1)	10^3	*Pseudomonas* sp. (2)	10^3	*Acremonium* sp. (2)
St. Serf's Church (indoor)						
S6	$<10^4$	nd	$<10^3$	nd	10^5	*Cladosporium cladosporioides* (6)
Stirling Castle (indoor)						
S1	10^3	*Streptomyces microflavus* (2)	10^5	*Pseudomonas anguilliseptica* (1)	10^4	*Acremonium furcatum* (2) *Penicillium* sp. (2)
S2	10^5	*Streptomyces microflavus* (2)	10^7	*Pseudomonas mandelii* (1) *Pseudomonas anguilliseptica* (2)	10^4	*Acremonium furcatum* (3)
S3	10^7	*Streptomyces microflavus* (1) *Streptomyces "vulgaris"* (1)	10^7	*Bacillus weihenstephanensis* (1) *Pseudomonas mandelii* (1) *Pseudomonas anguilliseptica* (2)	10^4	*Acremonium furcatum* (3)
S4	10^5	*Streptomyces microflavus* (13)	10^7	*Pseudomonas mandelii* (1) *Pseudomonas anguilliseptica* (2)	10^4	*Acremonium furcatum* (4)

Table 2. (*continued*)

Monument sample	Bacteria on AIA agar ($n=79$)		Bacteria on R2A agar ($n=59$)		Fungi ($n=76$)	
	cfu g^{-1}	Closest species (no of isolates)	cfu g^{-1}	Closest species (no of isolates)	cfu g^{-1}	Closest species (no of isolates)
S5	$<10^3$	nd	10^7	*Pseudomonas mandelii* (2) *Pseudomonas anguilliseptica* (2)	10^3	*Acremonium furcatum* (2)
Tolquhon Castle (outdoor)						
T1	10^6	*Streptomyces "vulgaris"* (2)	10^5	*Arthrobacter tumbae* (2) *Dyadobacter crusticola* (2)	$<10^3$	nd
T2	$<10^3$	nd	$<10^3$	nd	$<10^3$	nd
T3	10^6	*Streptomyces griseus* (1) *Streptomyces "vulgaris"* (2)	10^4	*Arthrobacter tumbae* (1) *Brevundimonas subvibrioides* (1) *Dyadobacter crusticola* (1)	$<10^3$	nd
T4	10^6	*Streptomyces griseus* (2) *Streptomyces laceyi* (1) *Streptomyces "vulgaris"* (6)	10^6	*Arthrobacter tumbae* (1) *Dyadobacter crusticola* (1)	10^5	*Acremonium/ Emericellopsis* sp. (2)

nd = no growth detected.

colonies, or more colonies representing all morphology types, were selected from the agar plates and subjected to preliminary screening procedures. The isolates were stored in glycerol at −70 °C.

Scanning electron microscopy

Samples for scanning electron microscopy (SEM) were air-dried, coated with gold (Balzers Union SCD 030) and examined in a Scanning Electron Microscope S-3200N (Hitachi, Nissei Sanyo, Japan) with an accelerating voltage of 18–20 kV [18].

Characterization of actinobacteria growing on AIA

The preliminary screening and characterization of actinobacterial isolates was performed using the automated ribotyping device RiboPrinter® System (DuPont Qualicon, Wilmington, DE, USA) with *Pvu*II as a restriction enzyme [50]. The mycelium was collected from cultures grown on cellulose nitrate filters of pore size 0.45 μm (Sartorius AG, Göttingen, Germany), placed onto the surface of the agar plate. The automated system generated a pattern for each strain using proprietary algorithms. Each batch included six marker lanes including a total of 30 molecular markers, which the system used for selection of a ribogroup already existing in the database or for creation of a new one and for calculation of the similarities between different patterns. A ribogroup is defined as a set of closely related patterns (threshold similarity 0.96) that are mathematically indistinguishable from one another by the system.

For identification, the databases of the manufacturer (Release 12.2 © 2000 Qualicon, update 2004) and one created at VTT during several research projects were used.

The chracterization of isolates representing different ribogroups (ribotypes) was continued by partial and almost complete (approximately 95%) 16S rRNA gene sequencing. Most sequences were carried out at the identification service of DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany). Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR product were carried out as described previously [44]. Purified PCR product was sequenced using the CEQ™DTCS-Quick Start Kit (Beckmann Coulter, Krefeld, Germany) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQ™8000 Genetic Analysis System (Beckmann Coulter). Primers 10.30f and 1525r were used for PCR and 530r, 357f, Foxf and 1100f for sequencing. The resulting sequence data was put into the alignment editor ae2 [35] and aligned manually. It was then compared with the 16S rRNA gene sequences of representative organisms belonging to the *Actinobacteria* [35]. The second set of sequences (marked with a star) was produced at VTT using the forward primers and technique described below. However, the sequences were compared using the same database at DSMZ.

In order to compare the riboprint patterns of isolates and those of relevant type strains (based on sequencing results), the patterns were transferred to the BioNumerics programme (Applied Maths, Sint-Martens-Latem, Belgium) and analysed by clustering methods using Pearson correlation and UPGMA. The streptomycetes

type strains were obtained from DSMZ and some type strains of other heterotrophic bacteria also from LMG (BCCM™/LMG bacteria collection, Gent, Belgium) and CIP (Institut Pasteur, collection of bacteria, Paris, France).

Characterization of other heterotrophic bacteria growing on R2A

Preliminary screening of the isolates was carried out on the basis of colony and cell morphology, Gram-staining, oxidase and catalase reaction and colour of pigments produced. Further screening of selected isolates was performed by RAPD fingerprinting using the random primers OPA-2 and OPA-3 [2]. Characterization of the isolates representing different RAPD types was continued by automated ribotyping using *Eco*RI as a restriction enzyme and by clustering the patterns in the BioNumerics programme as described above. The final identification of isolates was carried out by almost complete sequencing of the 16S rRNA gene in both directions. Total genomic DNA was obtained by lysing the cells mechanically using a FastPrep™ FP120 cell homogenizer (Savant Instruments, Inc., Holbrook, NY, USA). PCR was performed using primers BSF8/20 and BSR1541/20 [57] as described earlier [46]. Prior to sequencing, amplification products were purified using a QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Sequencing reactions of PCR products were performed with the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions using forward primers BSF8/20, BSF349/17, BSF1099/16 [57] and U772-f [41], and reverse primers BSR357/15, BSR534/18, BSR926/20, BSR1114/16 and BSR1541/20 [57]. Sequences were analysed with an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) and corrected manually (Chromas version 2.13, Technelysium, Australia). Sequences were aligned with DNAMAN software version 4.1 (Lynnon Biosoft, Canada). Similarity searching of sequences was performed using BLAST (NCBI) analysis [4].

Characterization of fungi

A stereomicroscope (Reichert-Jung, Polyvar, Wien, Austria) with a magnification of 400 × was used for preliminary screening of fungal isolates according to typical colonial and conidial morphology. Different fungal types were further characterized by sequencing targeting fungal Internal Transcribed Spacers (ITS). The PCR primers ITS1-F [13] and ITS4 [55] were used for PCR and sequencing. These primers amplify the highly variable ITS1 and ITS2 sequences surrounding the 5.8S-coding sequence situated between the Small SubUnit-coding sequence (SSU) and the Large SubUnit-coding sequence (LSU) of the ribosomal operon [55]. A wide range of fungal targets is addressed by these primers and a combination of ITS1-F and ITS4 works well in the examination of DNA isolated from individual organisms. Searches for most similar ITS1-5.8S-ITS2 regions in the GenBank database were performed using BLAST (NCBI) analysis [4]. Similarities between the respective sequences were calculated with ClustalW (EMBL-EBI) [26].

Results

SEM analysis

The original undisturbed biofilm observed in SEM revealed diverse microbial structures, which were developing in close contact with the material of the monuments (Figs. 1 and 2). All typical stone microbes (bacteria including filamentous actinobacteria, fungi and algae) were observed, although fungi and actinobacteria were the dominating microbial groups. The images of outdoor (Fig. 1) and indoor (Fig. 2) biofilms illustrated some differences between the biofilms developing on these sites. The outdoor samples revealed the presence of moniliform (bead-string-like, Fig. 1A) fungal hyphae and well developed EPS present in the intergranular spaces of the material. The protective environment of the extracellular exudate layer in an outdoor stone biofilm (Fig. 1C) harboured some heterotrophic components such as actinobacterial filaments (Figs. 1C and D). The green-brownish growth spots in the Stirling castle indoor sample consisted of diverse microbes associated with pennate, chain-forming diatom algae and formed an interconnected biofilm closely attached to the wall surface (Fig. 2). The indoor samples had a diverse and abundant growth of bacteria (Fig. 2A), diatom algae (Fig. 2B), as well as mycelial fungi (Fig. 2C) and actinobacteria (Fig. 2D). In both indoor and outdoor samples the microbial cells were frequently situated either between the grains of the mineral materials or below them or in surface depressions (Figs. 1A, C, D, 2A, C and D).

Actinobacteria on AIA agar

All isolates growing on AIA were streptomycetes and they were present in 17 of 20 samples (85%) in levels up to 10^5–10^6 cfu g^{-1} (Table 2). The most frequently occurring taxa were most closely related to *S. microflavus* (10 samples/3 monuments) or *S. "vulgaris"* (8/4). Among 79 isolates characterized by automated ribotyping, 21 different ribogroups (ribotypes) were obtained,

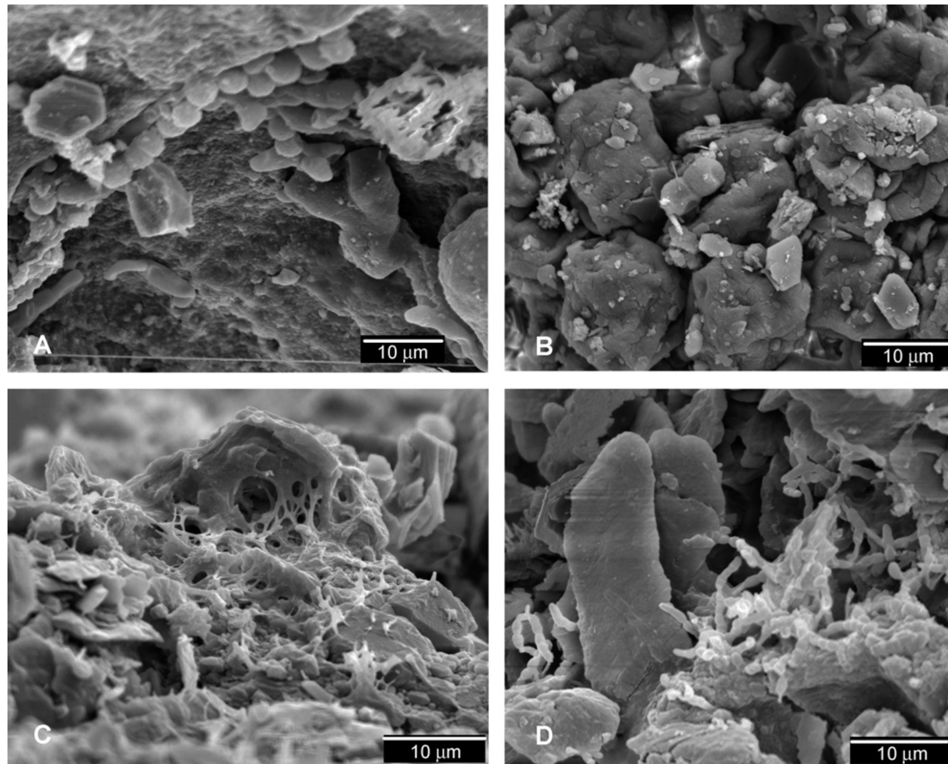

Fig. 1. Scanning electron microscopic images of outdoor biofilms of Linlithgow Palace ruins. (A) and (B) the presence of typical fungal growth forms in surface depressions. Moniliform (yeast-like) and cylindrical fungal filaments and bacterial cells growing on stone surface; (C) biofilm-associated slime or extracellular polymeric substances (EPS) developing under and between mineral particles of the substrate. Heterotrophic filaments of actinobacteria are probably also intermingled inside this protective slimy layer; (D) typical actinobacterial filaments developing between the mineral grains and in the deeper layers and cavities of the substrate.

from which only the riboprints of *S. microflavus* could be identified (similarity >0.85) with the riboprint databases used (data not shown), which was confirmed by clustering analyses (Fig. 3) and sequencing of the 16S rRNA gene (Table 3). Due to the clear results of ribotyping and partial 16S rRNA gene sequencing, only one of these isolates was almost complete sequenced. The type strain of *S. alboviridis* matched to the same ribogroup (Pvu-3) as the type strain of *S. microflavus* and some of our isolates. The 16S rRNA gene sequences of these isolates were also identical with those of *S. microflavus* and *S. alboviridis* (Table 3), indicating that these two currently different species cannot be differentiated by ribotyping with *Pvu*II or 16S rRNA gene sequencing. The same situation was observed for the species *S. atroolivaceus* and *S. olivoviridis*. The partial sequences of the other main group, most closely related to *S. ''vulgaris''* or *S. griseus*, were identical (100% to *S. griseus*). However, the almost complete sequences analysed, except for one, were slightly (one or two decimal points) more closely related to an undescribed species *S. ''vulgaris''* than to the type strain of *S. griseus* and to 10 other type strains, including e.g. *S. microflavus*, within the genus *Streptomyces*. The riboprints of this group were different from each other and did not cluster with the type strain of *S. griseus* or with the proposed type strain of *S. ''vulgaris''* (Fig. 3). The sequence of E-042644 was 99.9% identical to the type strains of *S. anulatus*, *S. cavourensis* subsp. *cavourensis*, *S. cavourensis* subsp. *washingtonensis*, *S. griseoplanus*, *S. praecox* and *S. scabiei*. However, its riboprint was quite different, only 0.21–0.40 to the riboprints of these type strains (data not shown).

Heterotrophic bacteria on R2A agar

Growth on R2A was present in 18 of 20 samples (90%) in levels up to 10^5–10^7 cfu g^{-1} (Table 2). Among the 59 isolates characterized by RAPD fingerprinting, 15 different RAPD profiles were detected (data not shown), from which one isolate (strain) per RAPD type was further analysed by ribotyping (Fig. 4) and by almost complete sequencing (Table 4). All isolates were oxidase- and catalase-positive, representing seven different genera. The most frequently detected isolates belonged to the genera *Arthrobacter* (12 samples/3 monuments) or *Pseudomonas* (9/3) (Table 2). The coccoid, from yellow to red pigmented *Arthrobacter* isolates occurred only in outdoor samples, whereas

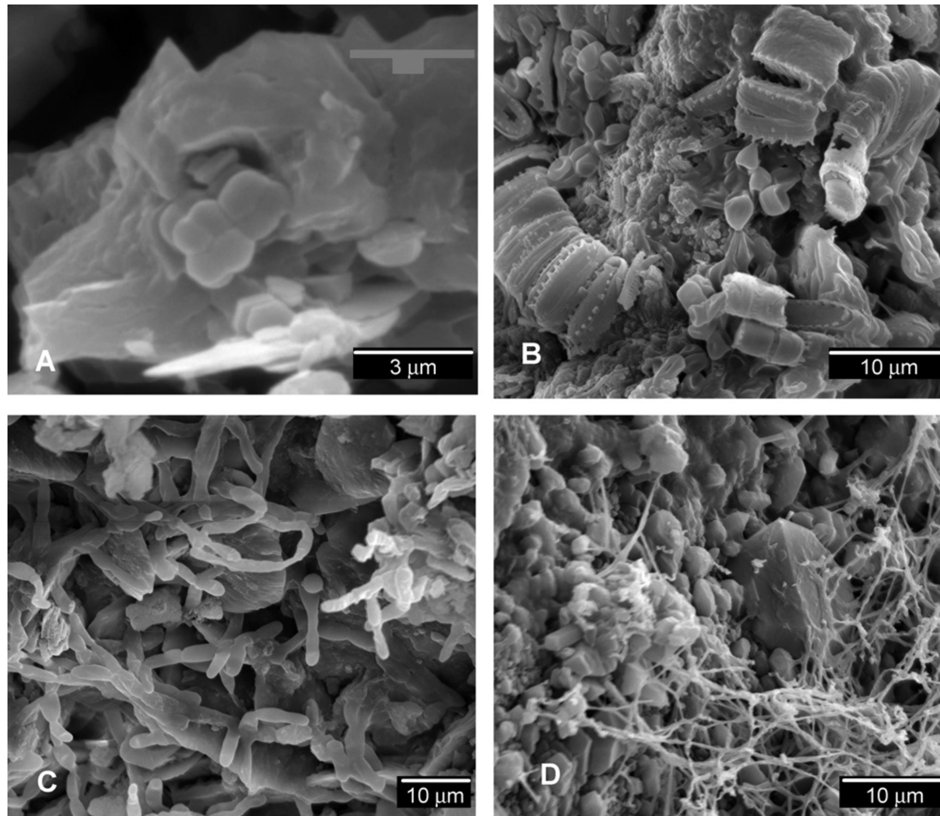

Fig. 2. Scanning electron microscopic images of indoor biofilms of Sterling Castle. (A) Mineral etching caused by coccoid bacteria (probably *Deinococcus*); (B) Pennate chain-forming diatom algae (oval- and round-shaped box-like symmetrical cells with a regular structure) with associated bacterial and fungal biofilm elements form an interconnected biofilm on the wall surface; (C) and (D) Heterotrophic filamentous components of the biofilm growth of fungi (C) and actinobacteria (D). Note the difference of 1/2 to 1 order of magnitude between the fungal and actinobacterial growth.

pseudomonads were common on indoor surfaces. The *Brevundimonas* isolate DNA could not be digested by *Eco*RI for ribotyping, which is typical for the strains of this genus, but it was digested by *Pvu*II. Its similarity to the closest type strain, *B. subvibrioides*, was only 0.07, indicating quite different fingerprints. Three of these strains (E-052916, E-052906, E-052914) had almost identical sequences (isolates) in the GeneBank (Table 4).

Fungal isolates

Fungi were isolated from 17 of 20 samples (85%) and their numbers were slightly lower than those of bacteria, being typically around 10^4–10^5 cfu g^{-1} (Table 2). A total of 76 fungal colonies were directly examined on agar plates by stereomicroscopy and 13 different morphological types were detected, of which 11 were characterized by ITS sequencing (Table 5). Hyaline *Acremonium* species (10 samples/4 monuments) were common in indoor samples, whereas the pigmented species of *Cladosporium* (8/3), *Penicillium* (6/3) and *Phialophora* (6/2) were typical for outdoor samples (Table 2). In addition, *Torrubiella* and *Trichothecium* isolates were typical for the outdoor samples of Arbroath Abbey.

Discussion

The studied biofilms indicated that different monuments have their own microbiota, although some similarities were also noticed. In this study, which focused on actinobacteria, other heterotrophic bacteria and fungi, the most frequently occurring bacterial genera were *Streptomyces*, *Arthrobacter* and *Pseudomonas* and the commonest fungal genera were *Cladosporium*, *Penicillium*, *Acremonium* and *Phialophora*. These genera have also been mentioned in previous articles considering stone (mineral) materials such as murals, paintings and caves [3,6,7,18,22,24,28,30,46]. However, identification of microbes to the species level is usually lacking. This is mainly due to the fact that these isolates are often members of hitherto undescribed species. In order to identify them, they should first be described as novel species. Based on the genetic information obtained in this study, many of these isolates, especially from the

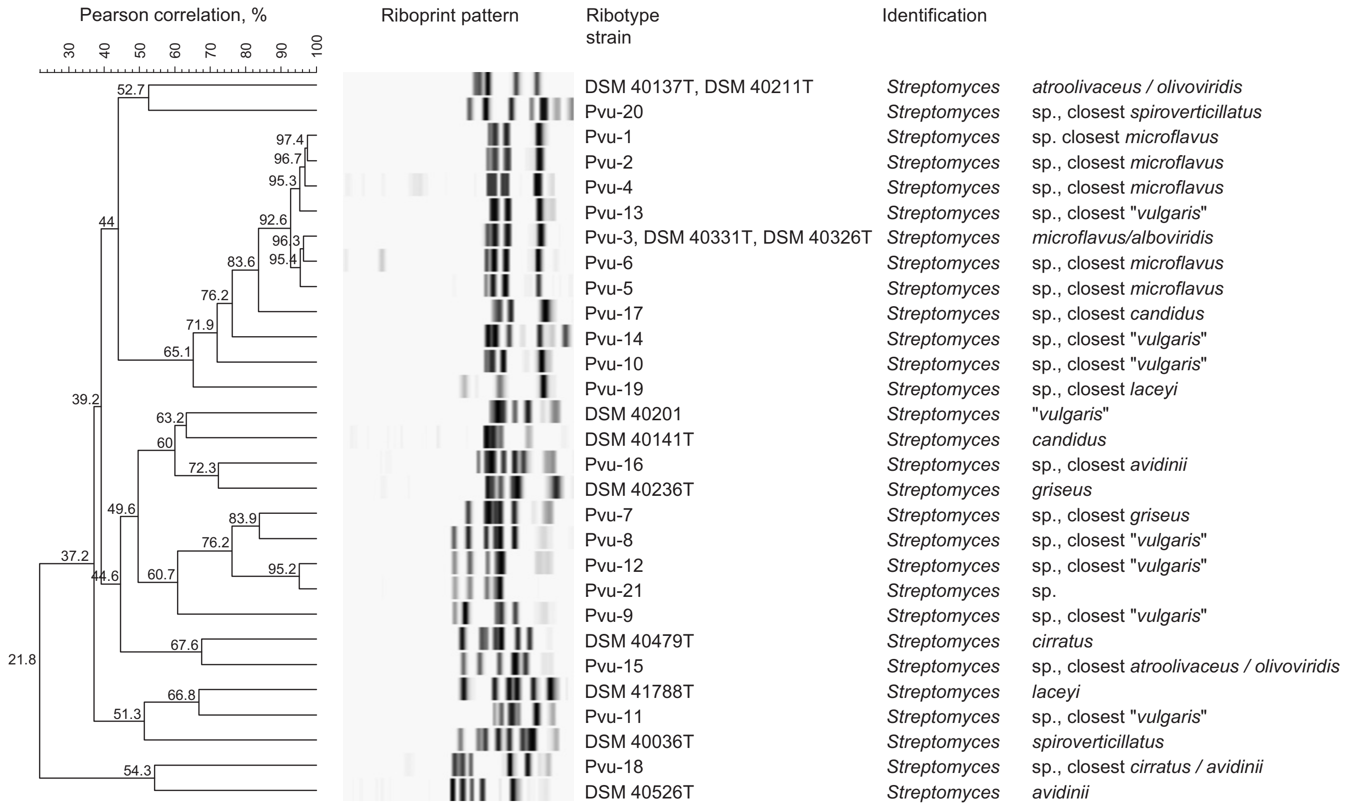

Fig. 3. Clustering of the generated riboprint patterns (*Pvu*II digestion) of different streptomycetes ribotypes (Table 3) and of 11 relevant type strains (*T*).

Table 3. Characterization of streptomycetes

Ribotype	Sample	Colour on AIA	Partial or almost complete 16S rRNA gene sequence			
			Strain	Length of sequence, nt	Closest similarity (%) to a type strain	
Pvu-1	Abbey 1, 2	Yellow-greenish	E-042969	790*	100	*S. microflavus/alboviridis*
Pvu-2	A1, A2, A3, A5, S1, S2	Yellow-greenish	E-042632	1491	100	*S. microflavus/alboviridis*
Pvu-3	A2, A3, S2, S4	Yellow-greenish	E-042399	744*	100	*S. microflavus/alboviridis*
Pvu-4	S3	Yellow-greenish	E-042648	756*	99.9	*S. microflavus/alboviridis*
Pvu-5	A3, S4	Yellow-greenish	E-042649	682*	100	*S. microflavus/alboviridis*
Pvu-6	S4	Yellow-greenish	E-042639	714*	100	*S. microflavus/alboviridis*
Pvu-7	T3, T4	Yellow-greenish	E-042007	1496	100	*S. griseus*
Pvu-8	Abbey 4	Cream-white	E-042670	1474*	100	*S. "vulgaris"*
Pvu-9	A3, A5	Cream-white	E-042660	1482*	100	*S. "vulgaris"*
Pvu-10	Abbey 4	Cream-white	E-042674	1480	100	*S. "vulgaris"*
Pvu-11	A3	Greyish	E-042638	1475	100	*S. "vulgaris"*
Pvu-12	T1, T4	Cream-white	E-042625	1472	99.7	*S. "vulgaris"*
Pvu-13	S3	White	E-042647	1477	100	*S. "vulgaris"*
Pvu-14	Abbey 3	White	E-042667	1495	99.9	*S. "vulgaris"*
Pvu-15	A1, A4	Cream-white	E-042628	1512	99.8	S. *atroolivaceus/olivoviridis*
Pvu-16	STA1	Cream-pink	E-052902	1492	99.8	*S. avidinii*
Pvu-17	Abbey 1	Cream-white	E-042677	1496	99.2	*S. candidus*
Pvu-18	Abbey 1	Cream-pink	E-052901	1511	99.8	*S. cirratus/avidinii*
Pvu-19	T4	White	E-042627	1494	98.9	*S. laceyi*
Pvu-20	STA1	Cream-pink	E-052903	1480	99.7	*S. spiroverticillatus*
Pvu-21	T4	White	E-042644	1509	99.9	*S.* species

*Sequence produced at VTT.

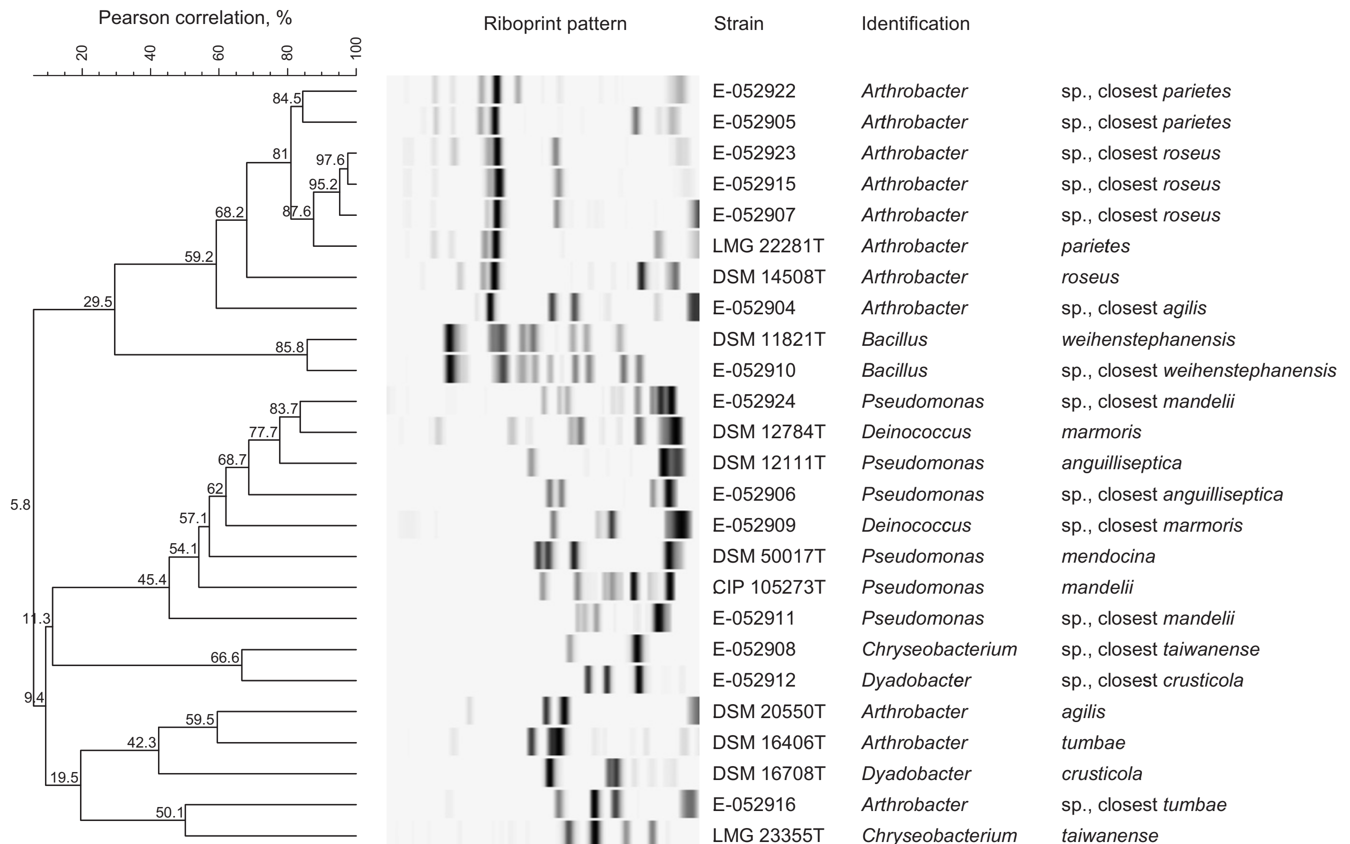

Fig. 4. Clustering of the generated riboprint patterns (*Eco*RI digestion) of different bacterial isolates (Table 4) and of 11 relevant type strains (*T*).

outdoor samples, can also be considered to represent potential members of novel species.

Although only a few characterizations were obtained at the high level of similarity (>99%), most of the isolates were well characterized at the genus level by almost complete 16S rRNA gene or ITS sequencing. Colony morphology, pigmentation and microscopy were in accordance with this genetic characterization. For the final identification, the physiological and chemo-taxonomical properties should also be studied. However, the environmental isolates adapted to highly specialized conditions and living in biofilms often change their typical species properties, which can hinder their phenotypic identification [51]. The identification of streptomycetes to the species level is difficult, since there is considerable overlap between results obtained with conventional (e.g. FAME profiles) and molecular methods (ribotyping, sequencing of the 16S rRNA gene) [32,50]. This was also observed in the present study, in which several sequences of the isolates were highly related to more than one type strain. In order to solve these taxonomic ambiguities, the next step in the identification of these isolates would be DNA/DNA hybridization with the closest type strains, which is laborious, expensive and out of reach for normal research projects. This situation makes routine identification of certain streptomycetes to the species level almost impossible, and calls for a reclassification of a large number of *Streptomyces* species. In this study, a close genetic relationship between *S. microflavus* and *S. alboviridis* was observed and it is possible that these names will in future be merged, hence e.g. *S. griseus* subsp. *cretosus*, *S. lipmanii* and *S. willmorei*, having identical riboprints with *S. alboviridis* in DuPont Qualicon database, have already been reclassified as *S. microflavus* [33]. In this study, we name our isolates of this taxon as *S. microflavus*, since it is an older name than *S. alboviridis*.

All bacteria detected on AIA agar were streptomycetes and their growth was also convincingly shown for *in situ* biofilms. AIA medium favours the growth of streptomycetes, although representatives of some other genera have also been successfully isolated on this medium [46,50]. Streptomycetes produce filamentous structures that aggressively penetrate into the substratum and promote the formation of biofilm on surfaces and microbial networks to considerable depths [54], which was also observed in this study. They are able to grow on very poor media and can exist for extended periods as resting arthrospores that germinate in the

Table 4. Characterization of heterotrophic bacteria from R2A agar

Strain	Sample	Gram	Cell morphology	Colour on R2A	Almost complete 16S rRNA gene sequence		
					Length of sequence, nt	Closest similarity (%) to a type strain (closest isolate)	
E-052904	Abbey 1	+	Coccus	Red	1522	99.7	*Arthrobacter agilis*
E-052916	A3	±	Coccus	Yellow	1516	97.8	*Arthrobacter tumbae* (99.9 *A. agilis* S23H2)
E-052905	Abbey 4	+	Coccus	Orange	1506	99.5	*Arthrobacter parietes*
E-052922	A5	+	Coccus	Orange	1419	99.6	*Arthrobacter parietes*
E-052907	A1	+	Coccus	Red	1509	98.3	*Arthrobacter roseus*
E-052915	A1	+	Coccus	Pink	1497	98.3	*Arthrobacter roseus*
E-052923	A5	+	Coccus	Red	1503	98.3	*Arthrobacter roseus*
E-052911	Abbey 3	–	Rod	Yellow	1523	99.5	*Pseudomonas mandelii*
E-052924	S5	–	Rod	Yellow	1526	99.5	*Pseudomonas mandelii*
E-052906	S2	–	Rod	Yellowish	1524	97.1	*Pseudomonas anguilliseptica* (99.3 *P. mendocina* PC1)
E-052910	S3	+	Rod	Beige	1531	100	*Bacillus weihenstephanensis*
E-052914	T3	+	Rod	Orange/ brown	1452	98.7	*Brevundimonas subvibrioides* (99.8 B. sp. V4.BP.05)
E-052908	Abbey 1	±	Short rod	Yellow/ orange	1508	96.3	*Chryseobacterium taiwanense*
E-052909	Abbey 4	+	Tetracoccus	Red/ orange	1505	95.7	*Deinococcus marmoris*
E-052912	T3	±	Short rod	Yellow	1493	98.0	*Dyadobacter crusticola*

Table 5. Characterization of fungi

Strain	Sample	Macroscopic morphology	ITS1-5.8S-ITS2 sequence		
			Length of sequence, nt	Similarity (%) to a species	
D-041030	S2	Whitish, exudate droplets, flat	466	98.1	*Acremonium furcatum*
D-041031	S3	Whitish, slimy, glossy, thorns	464	97.4	*Acremonium furcatum*
D-041032	S4	Whitish, clumpy, exudate droplets	467	98.5	*Acremonium furcatum*
D-041035	T4	White, exudate droplets	482	90.5	*Emericellopsis pallida*/90.2% *Acremonium potronii*
D-041033	S6	Black greenish, penetrates into agar	458	100	*Cladosporium cladosporioides*
D-041034	S6	Black greenish, penetrates into agar	458	100	*Cladosporium cladosporioides*
D-041028	A4	Dark green, large colonies	459	100	Many *Cladosporium* species
D-041027	A5	White, cotton-like, reverse orange	na	na	*Fusarium* sp. (morphological identification)
D-041024	Abbey 1	Green, brownish reverse	na	na	*Penicillium* sp. (morphological identification)
D-041023	Abbey 1	Small black colony, clumpy	534	83.0	*Phialophora lignicola*
D-041026	Abbey 4	Small black colony, clumpy	534	83.0	*Phialophora lignicola*
D-041025	Abbey 4	White, fasciculated	496	100	*Torrubiella confragosa*
D-041022	Abbey 2	Orange, hairy, yeast-like	465	94.9	*Trichotecium domesticum*

na = not analyzed.

occasional presence of nutrients [17]. It is proposed that these bacteria could be the primary colonizers of frescoes located in relatively humid hypogeal environments such as tombs, crypts and grottoes [24]. The partial 16S rRNA gene sequences of the other dominant actinobacterial taxa, especially typical to Tolquhon Castle samples, were 100% related to the type strain of *S. griseus* and to each other, whereas the complete sequences pointed slightly closer to an undescribed species *S.* "*vulgaris*". Genetically similar bacteria resembling *S. griseus* have earlier been isolated from building materials of water-damaged houses [52]. Thus, these environmental isolates could represent one or more separate species closely related to *S. griseus*.

Almost all bacteria isolated on R2A agar were yellow- or red-pigmented and were mainly members of the genus *Arthrobacter*. Taxonomically these bacteria belong to the actinobacteria. Their presence has been reported in many cultural heritage sites, they have been proposed to be the first colonizers of murals [42], and recently six novel species isolated from deteriorated mural paintings were described [25]. Their co-existence with streptomycetes in the same biofilms is feasible, because their cell walls are not lysed by the exoenzymes of streptomycetes [30]. Isolates most closely related to *A. agilis* were typical to Arbroath Abbey, whereas isolates most closly related to *A. parietes* and *A. roseus* were typical to Linlithgow Palace. Isolates typical to Tolquhon Castle were most closely related (97.8%) to the type strain of *A. tumbae*, although much higher similarities were detected in GeneBank to psychrophilic isolates S23H2 (99.9%) and LV7 (99.7%) both isolated in Antarctica, growing at 0–5 °C and considered at that time to be most closely related to *A. agilis* [29,34]. All these three strains are genetically relatively far from the type strains and could represent a new species.

Only pseudomonads and one *Bacillus* isolate were isolated from the indoor samples of Stirling Castle. Among pseudomonads there were isolates with sequence similarities most closely related to *P. mendocina* isolate PC1 (99.3%) [37]. However, the closest type strain was *P. anguilliseptica* (97.1%), indicating again an unknown typical stone species. *Bacillus* species are common as secondary biofilm formers in environmental biofilms [7,15,24]. The sporadic detection of bacilli from an indoor sample in this study may be due to the fact that the R2A agar used was unsuitable, e.g. lacking salts required, for the growth of wall-bacilli, or more probably that the outdoor stones are too poor an environment for these bacteria. A novel species within this genus, *B. herbersteinensis*, was recently isolated from a medieval wall painting in a chapel [56].

Chryseobacterium and *Deinococcus* isolates only occurred in biofilms of Arbroath Abbey, whereas *Brevundimonas* and *Dyadobacter* isolates were only present in biofilms of Tolquhon Castle. Deinococci were most closely related to *D. marmoris*, but the similarity level was low, only 95.7%, strongly indicating a novel species. This is a very interesting observation, because another *Deinococcus*, *D. geothermalis*, is recognized to be a good primary biofilm former on hard surface materials such as stainless steel [31]. Recently, three novel *Deinococcus* species isolated from Antarctic soil and rock samples [27], and nine isolated from arid soil [43] were described. These deinococci are considered to be low temperature-tolerating, draught-tolerating and UV-resistant bacteria. In this study, only two deinococci isolates were detected by cultivation from outdoor samples. However, similar tetracocci were also observed *in situ* in an indoor sample (Fig. 2A). The low number of isolates observed may be due to the growth temperature applied (25 °C), which was perhaps too high for their growth because the optimum temperature of these bacteria is reported to be as low as from 9 to 16 °C [27]. It is also known that these bacteria are detected only on nutrient-poor growth media [31], which indicates that they are likely to inhabit nutrient-limited materials such as stone. The almost complete sequence of our *Brevundimonas* isolate was very close related (99.8%) to *Brevundimonas* sp. V4.BP.05, isolated from the Mediterranean Sea [12]. However, the closest type strain was *B. subvibrioides*, with relatively low similarity (98.7%). In addition, the detected yellow isolates of *Dyadobacter* could potentially be novel colonizers of stone materials, although little is currently known about these bacteria. They are known to be adapted to live in extreme conditions on biological soil crusts and to be psychrotolerant, growing at temperatures as low as 5 °C [45]. The use of lower incubation temperatures, e.g. 15 °C, and dilution of the commercial R2A medium used might have resulted in the isolation of more of these extremely cold-tolerant and low-nutrient isolates. Thus, the occurrence of these bacteria on stone surfaces in open air may be more common than indicated in this study.

Filamentous fungi are mainly known to be responsible for the decomposition of wooden materials. However, due to their growth forms and high adaptive potential, they are also abundant and important microbes in the colonization and biodeterioration of stone materials [36,49,53]. Fungi are an order of magnitude larger than bacteria at similar levels of metabolic activity and therefore represent a considerably higher deteriorative potential than bacteria. The fungus *Cladosporium cladosporioides* is widespread in nature [48] and its spores are common in air especially in autumn, when they may cause allergic responses in humans. *Phialophora* species have been isolated from mineral surfaces [6,46] and are known to occur in nutrient-poor environments. *Torrubiella* is an insect and spider parasite [47], which has earlier been isolated e.g. from biodeteriorated surfaces of Roman catacombs [46].

Its repeated isolation from the samples of Abroath Abbey is probably associated with the remains of microscopic animals grazing or dwelling on the wall surfaces. *Trichothecium, Acremonium* and *Penicillium* represent common soil and air isolates. It is possible that some of the very slowly growing members of the black fungi, known as persistent inhabitants of rock surfaces, escaped our attention, because their isolation may need special techniques [40].

In general, the investigated biofilms were shown to contain site-specific actively growing microbial communities developing on the surfaces of the studied monuments. These biofilms had a very close contact with the substrate and thus they probably exercise considerable deteriorative effects on the underlying materials. Knowledge of the specific microbiota of target sites will facilitate the development of most suitable preventive measures [1,58].

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References

[1] H.-L. Alakomi, A. Paananen, M.-L. Suihko, I.M. Helander, M. Saarela, Weakening effect of cell permeabilizers on Gram-negative bacteria causing biodeterioration, Appl. Environ. Microbiol. 72 (2006) 4695–4703.

[2] M. Alander, J. Mättö, W. Kneifel, M. Johansson, B. Kögler, R. Crittenden, T. Mattila-Sandholm, M. Saarela, Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract, Int. Dairy J. 11 (2001) 817–825.

[3] P. Albertano, C. Urzi, Structural interactions among epilithic cyanobacteria and heterotrophic microorganisms in Roman hypogea, Microb. Ecol. 38 (1999) 244–252.

[4] S.F. Altschul, T.L. Madden, A.A. Schäffer, J. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.

[5] E. Bock, W. Sand, A review: the microbiology of masonry biodeterioration, J. Appl. Bacteriol. 74 (1993) 503–514.

[6] J. Braams, Ecological studies on the fungal microflora inhabiting historic sandstone monuments, Ph.D. Thesis, Geomicrobiology, Oldenburg University, Germany, 1992.

[7] O. Ciferri, Microbial degradation of paintings, Appl. Environ. Microbiol. 65 (1999) 879–885.

[8] E. Diakumaku, A.A. Gorbushina, W.E. Krumbein, L. Panina, S. Soukharjevski, Black fungi in marble and limestones – an aesthetical, chemical and physical problem for the conservation of monuments, Sci. Total Environ. 167 (1995) 295–304.

[9] T. Dornieden, A.A. Gorbushina, W.E. Krumbein, Änderungen der physikalischen Eigenschaften von Marmor durch Pilzbewuchs, Int. J. Restor. Buildings Monuments 3 (1997) 441–456.

[10] Th. Dornieden, A.A. Gorbushina, W.E. Krumbein, Biodecay of cultural heritage a space/time-related ecological situation – an evaluation of a series of studies, Int. Biodeterior. Biodegrad. 46 (2000) 261–270.

[11] N.J.C. Fong, M.L. Burgess, K.D. Barrow, Carotenoid accumulation in the psychrotrophic bacterium *Arthrobacter agilis* in response to thermal and salt stress, Appl. Microbiol. Biotechnol. 56 (2001) 750–756.

[12] I. Fritz, C. Strömpl, D.I. Nikitin, A.M. Lysenko, W.-R. Abraham, *Brevundimonas mediterranea* sp. nov., a non-stalked species from the Mediterranean Sea, Int. J. Syst. Evol. Microbiol. 55 (2005) 479–486.

[13] M. Gardes, T.D. Bruns, ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts, Mol. Ecol. 2 (1993) 113–118.

[14] C.C. Gaylarde, L.H.G. Morton, Deteriogenic biofilms on buildings and their control: a review, Biofouling 14 (1999) 59–74.

[15] I. Gonzalez, L. Laiz, B. Hermosin, B. Caballero, C. Incerti, C. Saiz-Jimenez, Bacteria isolated from rock art paintings: the case of Atlanterra shelter (south Spain), J. Microbiol. Meth. 36 (1999) 123–127.

[16] J.M. Gonzalez, C. Saiz-Jimenez, Research review: application of molecular nucleic acid–base techniques for the study of microbial communities in monuments and artwork, Int. Microbiol. 8 (2005) 189–194.

[17] M. Goodfellow, S.T. Williams, Ecology of actinomycetes, Annu. Rev. Microbiol. 37 (1983) 189–216.

[18] A.A. Gorbushina, J. Heyrman, T. Dornieden, M. Gonzalez-Delvalle, W.E. Krumbein, L. Laiz, K. Petersen, C. Saiz-Jimenez, J. Swings, Bacterial and fungal diversity and biodeterioration problems in mural painting environments of St. Martins church (Greene-Kreiensen Germany), Int. Biodeterior. Biodegrad. 53 (2004) 13–24.

[19] A.A. Gorbushina, W.E. Krumbein, C.-H. Hamann, L. Panina, S. Soukharjevski, U. Wollenzien, Role of black fungi in color change and biodeterioration of antique marbles, Geomicrobiol. J. 11 (1993) 205–221.

[20] A.A. Gorbushina, K. Petersen, Distribution of microorganisms on ancient wall paintings as related to

associated faunal elements, Int. Biodeterior. Biodegrad. 46 (2000) 277–284.

[21] P.S. Griffin, N. Indictor, R.J. Koestler, The biodeterioration of stone: a review of deterioration mechanisms, conservation case histories and treatment, Int. Biodeterior. 28 (1991) 187–207.

[22] I. Groth, R. Vettermann, B. Schuetze, P. Schumann, C. Saiz-Jimenez, Actinomycetes in Karstic caves of northern Spain (Altamira and Tito Bustillo), J. Microbiol. Meth. 36 (1999) 115–122.

[23] C. Gurtner, J. Heyrman, G. Pinar, W. Lubitz, J. Swings, S. Rölleke, Comparative analyses of the bacterial diversity on two different biodeteriorated wall paintings by DGGE and 16S rDNA sequence analysis, Int. Biodeterior. Biodegrad. 46 (2000) 229–239.

[24] J. Heyrman, J. Swings, 16S rDNA sequence analysis of bacterial isolates from biodeteriorated mural paintings in the Servilia tomb (Necropolis of Carmona, Seville, Spain), Syst. Appl. Microbiol. 24 (2001) 417–422.

[25] J. Heyrman, J. Verbeeren, P. Schumann, J. Swings, P. De Vos, Six novel *Arthrobacter* species isolated from deteriorated mural paintings, Int. J. Syst. Evol. Microbiol. 55 (2005) 1457–1464.

[26] D. Higgins, J. Thompson, T. Gibson, J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position-specific gap penalties and weight matrix choice, Nucleic Acids Res. 22 (1994) 4673–4680.

[27] P. Hirsch, C.A. Gallikowski, J. Siebert, K. Peissl, R. Kroppenstedt, P. Schumann, E. Stackebrandt, R. Anderson, *Deinococcus frigens* sp. nov., *Deinococcus saxicola* sp. nov., and *Deinococcus marmoris* sp. nov., low temperature and draught-tolerating, UV-resistant bacteria from continental Antarctica, System. Appl. Microbiol. 27 (2004) 636–645.

[28] I. Ionita, Contributions to the study of the biodeterioration of the work of art and of historic monuments. II. Species of fungi involved in the deterioration of mural paintings from the monasteries of Moldavia, Revue Roumaine de Biologie. Series de Botanique 18 (1973) 179–189.

[29] K. Junge, J.J. Gosink, H.-G. Hoppe, J.T. Staley, *Arthrobacter*, *Brachybacterium* and *Planococcus* isolates identified from Antarctic Sea ice brine. Description of *Planococcus mcmeekinii*, sp. nov. System, Appl. Microbiol. 21 (1998) 306–314.

[30] N. Karpovich-Tate, N.L. Rebrikova, Microbial communities on damaged frescoes and building materials in the cathedral of the nativity of the virgin in the Pafnutii-Borovskii monastery, Russia, Int. Biodeterior. 27 (1990) 281–296.

[31] M. Kolari, Attachment mechanisms and properties of bacterial biofilms on non-living surfaces, Ph.D. Thesis, Department of Applied Chemistry and Microbiology, Division of Microbiology, University of Helsinki, Finland, 2003.

[32] B. Lanoot, M. Vancanneyt, B. Hoste, K. Vandemeulebroecke, M.C. Cnockaert, P. Dawyndt, Z. Liu, Y. Huang, J. Swings, Grouping of streptomycetes using 16S-ITS RFLP fingerprinting, Res. Microbiol. 156 (2005) 755–762.

[33] B. Lanoot, M. Vancanneyt, A. Van Schoor, Z. Liu, J. Swings, Reclassification of *Streptomyces nigrifaciens* as a later synonym of *Streptomyces flavovirens*; *Streptomyces citreofluorescens*, *Streptomyces chrysomallus* subsp. *chrysomallus* and *Streptomyces fluorescens* as later synonyms of *Streptomyces anulatus*; *Streptomyces chibaensis* as a later synonym of *Streptomyces corchorusii*; *Streptomyces flaviscleroticus* as a later synonym of *Streptomyces minutiscleroticus*; and *Streptomyces lipmanii*, *Streptomyces griseus* subsp. *alpha*, *Streptomyces griseus* subsp. *cretosus* and *Streptomyces willmorei* as later synonyms of *Streptomyces microflavus*, Int. J. Syst. Evol. Microbiol. 55 (2005) 729–731.

[34] J. Loveland-Curtze, P.P. Sheridan, K.R. Gutshall, J.E. Brenchley, Biochemical and phylogenetic analyses of psychrophilic isolates belonging to the *Arthrobacter* subgroup and description of *Arthrobacter psychrolactophilus*. sp. nov., Arch. Microbiol. 171 (1999) 355–363.

[35] B.L. Maidak, J.R. Cole, C.T. Parker Jr., G.M. Garrity, N. Larsen, B. Li, T.G. Lilburn, M.J. McCaughey, G.J. Olsen, R. Overbeek, S. Pramanik, T.M. Schmidt, J.M. Tiedje, C.R. Woese, A new version of the ribosomal database project (RDP), Nucleic Acids Res. 27 (1999) 171–173.

[36] E. May, F.J. Lewis, S. Pereira, S. Tayler, M.R.D. Seaward, D. Allsopp, Microbial deterioration of building stone – a review, Biodeterior. Abstr. 7 (1993) 109–123.

[37] M. Merimaa, E. Heinaru, M. Liivak, Grouping of phenol hydroxylase and catechol 2,3-dioxygenase genes among phenol- and *p*-cresol-degrading *Pseudomonas* species and biotypes, Arch. Microbiol. 186 (2006) 287–296.

[38] R. Mitchell, J.D. Gu, Changes in the biofilm microflora of limestone caused by atmospheric pollutants, Int. Biodeterior. Biodegrad. 46 (2000) 299–303.

[39] R. Moeller, G. Horneck, R. Facius, E. Stackebrandt, Role of pigmentation in protecting *Bacillus* sp. endospores against environmental UV radiation, FEMS Microbiol. Ecol. 51 (2005) 231–236.

[40] P. Möhlenhoff, L. Müller, A.A. Gorbushina, K. Petersen, Molecular approach to the characterisation of fungal communities: methods for DNA extraction, PCR amplification and DGGE analysis of painted art objects, FEMS Microbiol. Lett. 195 (2001) 169–173.

[41] M.A. Nadkarni, F.E. Martin, N.A. Jacques, N. Hunter, Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set, Microbiology 148 (2002) 257–266.

[42] J.P. Petushkova, N.N. Lyalikova, Microbiological degradation of lead-containing pigments in mural paintings, Stud. Conserv. 31 (1986) 65–69.

[43] F.A. Rainey, K. Ray, M. Ferreira, B.Z. Gatz, M.F. Nobre, D. Bagaley, B.A. Rash, M.-J. Park, A.M. Earl, N.C. Shank, A.M. Small, M.C. Henk, J.R. Battista, P. Kämpfer, M.S. da Costa, Extensive diversity of ionizing-radiation-resistant bacteria recovered from Sonoran Desert soil and description of nine new species of the genus *Deinococcus* obtained from a single soil sample, Appl. Environ. Microbiol. 71 (2005) 5225–5235.

[44] F.A. Rainey, N. Ward-Rainey, R.M. Kroppenstedt, E. Stackebrandt, The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage; proposal of *Nocardiopsaceae* fam. nov., Int. J. Syst. Bacteriol. 46 (1996) 1088–1092.

[45] G.S.N. Reddy, F. Garcia-Pichel, *Dyadobacter crusticola* sp. nov., from biological soil crusts in the Colorado Plateau, USA, and an amended description of the genus *Dyadobacter* Chelius and Triplett 2000, Int. J. Syst. Evol. Microbiol. 55 (2005) 1295–1299.

[46] M. Saarela, H.-L. Alakomi, M.-L. Suihko, L. Maunuksela, L. Raaska, T. Mattila-Sandholm, Heterotrophic microorganisms in air and biofilm samples from Roman catacombs with a special emphasis on actinobacteria and fungi, Int. Biodeterior. Biodegrad. 54 (2004) 27–37.

[47] R.A. Samson, H.C. Evans, J.-P. Latge (Eds.), Atlas of Entomopathogenic Fungi, Springer, Heidelberg, 1988.

[48] M.A. Shirakawa, I.B. Beech, T. Tapper, M.A. Cincotto, W. Gambale, The development of a method to evaluate bioreceptivity of indoor mortar plastering to fungal growth, Int. Biodeterior. Biodegrad. 51 (2003) 83–97.

[49] K. Sterflinger, W.E. Krumbein, Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones, Geomicrob. J. 14 (1997) 219–222.

[50] M.-L. Suihko, R.M. Kroppenstedt, E. Stackebrandt, Occurrence and characterization of actinobacteria and thermoactinomycetes isolated from pulp and board samples containing recycled fibres, J. Ind. Microbiol. Biotechnol. 33 (2006) 183–191.

[51] M.-L. Suihko, E. Stackebrandt, Identification of aerobic mesophilic bacilli isolated from board and paper products containing recycled fibres, J. Appl. Microbiol. 1 (2003) 25–34.

[52] M. Suutari, E. Rönkä, U. Lingnell, H. Rintala, A. Nevalainen, Characterisation of *Streptomyce*s spp. isolated from water-damaged buildings, FEMS Microbiol. Ecol. 39 (2002) 77–84.

[53] H.A. Viles, A.A. Gorbushina, Soiling and microbial colonisation on urban roadside limestone: a three year study in Oxford, England, Build. Environ. 38 (2003) 1217–1224.

[54] T. Warscheid, J.M. Braams, Biodeterioration of stone: a review, Int. Biodeterior. Biodegrad. 46 (2000) 343–368.

[55] T.J. White, T. Bruns, S. Lee, J.W. Taylor, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocols: A Guide to Methods and Applications, Academic Press Inc, New York, 1990, pp. 315–322.

[56] M. Wieser, H. Worliczek, P. Kämpfer, H.-J. Busse, *Bacillus herbersteinensis* sp. nov., Int. J. Syst. Evol. Microbiol. 55 (2005) 2119–2123.

[57] A. Wilmotte, G. Van der Auwera, R. De Wachter, Structure of the 16S ribosomal RNA of the thermophilic cyanobacterium *Chloroglaeopsis* HTF (*Mastigocladus laminosus* HTF) strain PCC7518, and phylogenetic analysis, FEBS Lett. 317 (1993) 96–100.

[58] M.E. Young, H.-L. Alakomi, I. Fortune, A. Gorbushina, W.E. Krumbein, I. Maxwell, C. McCullagh, P. Robertson, M. Saarela, J. Valero, M. Vendrell, Development of a biocidal treatment regime to inhibit biological growths on cultural heritage: BIODAM, Building Environ. 2006, in press.