MICROBIAL ENDOLITHIC BIOFILMS: A MEANS OF SURVIVING THE HARSH CONDITIONS OF THE ANTARCTIC

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ABSTRACT

Much of the Antarctic continent's microbiota is restricted to endolithic microecosystems which harbour distinct microbial communities as biofilms. The lithic substrate and the microorganisms comprising these films are intimately linked, giving rise to complex mineral-microbe interactions. The Antarctic biofilms analysed in this study were characterised by the presence of extracellular polymer substances. Cyanobacteria appeared as key components of these biofilms in zones where there were no nearby lichen thalli. Fungal cells were the predominant organisms in areas inhabited by epilithic lichens. The combined use of microscopy and molecular techniques enabled the identification of the different biological components of biofilms found in subsurface layers of the lithic substrate. It is proposed that in this extreme environment, the structure of the biofilm may favour the formation of microsites with specific physicochemical conditions that permit the survival of microbial communities.

1. INTRODUCTION

Antarctic ecosystems are among the most interesting extreme environments available for microbial diversity and ecological studies related to astrobiology. Terrestrial life in the Antarctic continent is limited by harsh environmental conditions during most of the year. Some microorganisms are capable of living in these conditions by colonising internal zones of the rock. Thus, we may find microbial life in areas where the environmental conditions prevent colonisation of rock surfaces [1]. If we are to understand how these microorganisms are able to survival these extreme conditions, we first need to establish the physical organisation of integrated communities within local microenvironments. This, however, is a difficult task because of the minute, dynamic nature of the space to be analysed, and few endolithic communities have been extensively explored in natural conditions. Notwithstanding, the ideal tool for this purpose is microscopy, especially when applied *in situ,* that is, when the biological components are not separated from the lithic material that arrives at the laboratory [2,3,4].

The present report describes an integrated study of the biological and structural components of the endolithic microhabitat based on the combined use of several microscopy techniques, *in situ*, including scanning electron microscopy in back scattered electron mode (SEM-BSE), confocal laser scanning microscopy (CSLM) and transmission electron microscopy (TEM).

2. MATERIAL AND METHODS

Pieces of granite rock with subsurface layers containing endolithic microorganisms were collected from the Ross Sea coast. The rock samples were obtained under natural conditions and stored at –20ºC until processing for microscopy or microanalytical procedures.
Cyanobacteria were prepared for TEM by removal from the rock and blocking in agar. Small pieces containing the cyanobacterial cells were fixed in gluteraldehyde and osmium tetroxide solutions, dehydrated in ethanol series, and embedded in Spurr´s resin following the protocol described in [5]. Ultrathin sections were post-stained with lead citrate and observed using a Philips 300 transmission electron microscope.
Lichen-rock cross sections were observed by SEM-BSE and CSLM after preparing samples according to a previously described procedure [6]. In brief, hand-cut pieces of rock were first fixed in glutaraldehyde and then in osmium tetroxide (only for SEM-BSE). This was followed by dehydration in a graded series of

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ethanol solutions and embedding in LR-white resin. Blocks of resin-embedded rock samples were finely polished and subsequently observed using a confocal laser Zeiss LSM310 microscope or scanning electron DMS 960-Zeiss microscope. Microprobe analyses were performed by energy dispersive spectroscopy (EDS) by applying a Link ISIS microanalytical system during the SEM observations.

3. RESULTS AND DISCUSSION

Endolithic microecosystems harbour distinct microbial communities as biofilms. The confocal microscopy images (Fig. 1) show these communities embedded in a polymeric matrix. Note the presence of a bacterial colony within the polymeric matrix inside a rock fissure. The three dimensional images provided by this technique reveal the spatial organisation of the biofilm's components and also give an idea of the number of microorganisms present [7]. In some rocks, these cells and matrices completely filled existing fissures.

The biofilms were comprised of different microorganisms. In some areas, cyanobacterial colonies were the main components of the hypolithic biofilm (Fig. 2) and could also be observed in internal fissures among mica layers (Fig. 3). Using the SEM-BSE technique, the ultrastructural and morphological details of cells embedded in the rock can be discerned. These cells show different types of sheaths which can be more easily compared using TEM. In some groups of cells, sheaths comprised of two different layers could be distinguished: an outer layer of concentric agglutinations of fibres (I) and an inner, less electro-dense layer with thinner and more irregularly distributed fibres (II) (White arrow in Fig. 4). Some groups of cells were enveloped only by the former type of layer (Black arrow in Fig. 4). Correlating these differences with their position in the rock by means of SEM-BSE has led to speculation about the existence of different physiological stages or levels of adaptation to the environment inside the biofilm [7].

While cyanobacteria appeared as key components of biofilms in zones where there were no lichen thalli nearby, fungal and algal cells were the predominant organisms in zones where epilithic lichens were present or fruiting bodies of endolithic lichens could be externally observed (Fig. 5).

Despite microscopy being essential for understanding biofilm structure and mineral-microorganism interactions, for identifying microorganisms we need to go beyond simply observing them. This means that microscopy has to be combined with molecular techniques, though such methods need to be adapted to working with endolithic communities. Samples are small but this makes possible to differentiate microorganisms close to each other. We isolated DNA from a small endolithic mass associated with the rock. By subsequent PCR using specific primers and sequencing of the PCR products, it was possible to identify the microbial components of the biofilm. Using these methods it has already been possible to observe the presence of different genera of cyanobacteria and epilithic and endolithic lichenised fungi (De los Ríos and Grube, unpublished).

An analysis of the complete cycle of endolithic life in the harsh, unstable Antarctic habitat would not be complete without considering decay and fossilisation processes. Biofilms containing only a few live cells have been frequently observed in deep fissures. Cell and sheath remnants intermix with live or mummified cells. These structures are highly variable since mummified cells are unstable. Thus, it is necessary to search for traces left behind by microorganisms after their death, as signs of previous life. In some fissures, cells were seen to be surrounded by mineral deposits which may eventually become biomarkers of these cells after their disintegration (Fig. 6). Finally, fossils of microorganisms that formed part of endolithic biofilms have also been found in Antarctica [4,8,9,10]. These mineralised structures show ultrastructural details such that their biological origin can be clearly inferred by the expert eye.

Besides the organisation and identification of the biofilm's components, the microenvironments within Antarctic biofilms need to be characterised. The lithic substrate and microorganisms are intimately linked, generating complex mineral-microbe interactions that give rise to different chemical microenvironments. Some zones of the substrate could be seen to be highly altered by the EDS microanalysis system chosen to evaluate the chemical processes that occur in these areas. Changes in the chemical composition of the mineral substrate (biomobilisation processes) are frequently observed in association with altered areas [8,9]. On occasion, the products of biomineralisation can also be identified. Figure 7 shows spatial distribution maps of the elements Si and Ca obtained by EDS in a fissure zone. Calcium deposition can be seen in the vicinity of the mineral substrate and can be related to the formation of calcium oxalate precipitates. In addition, distinctly acidic environments in the proximity of endolithic communities have been detected by confocal microscopy and Cl-NERF staining, pH values lower than 3.5 being registered close to cells [7]. The presence of acidic extracellular polymers together with the production of inorganic and organic acids could account for the acid pH registered. The low pH of these microenvironments can clearly affect the development of the communities. Microorganisms affect mineralogical processes and vice-versa. They participate in weathering reactions and the resulting weathering action on the substrate in turn conditions the formation of certain microhabitats.

In conclusion, these endolithic biofilms can be viewed as systems immobilised in rock enveloped by an organic matrix resulting from the excretion of exopolysaccharides by resident microorganisms. The physical and chemical conditions of a biofilm may vary and are often much less severe than those of the external environment. Thus, microorganisms within the biofilm structure may be protected from the environment.

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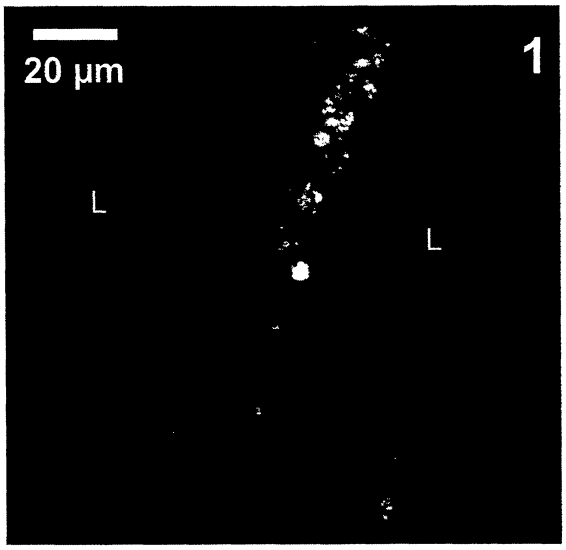


Fig. 1. CSLM image of a Lucifer yellow-stained specimen showing a microbial community embedded in a polymeric matrix. L: lithic substrate.

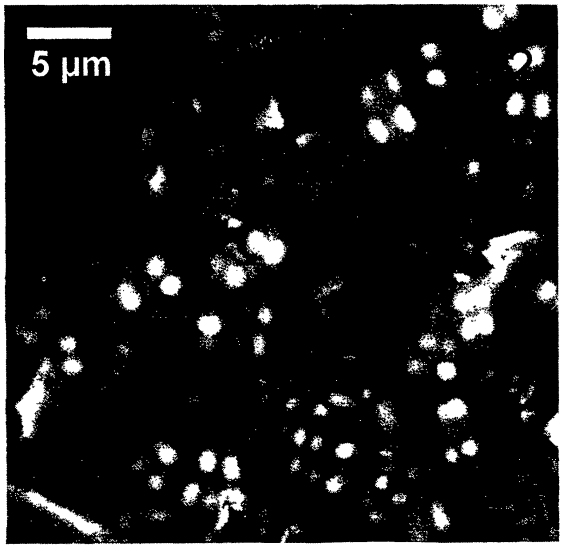
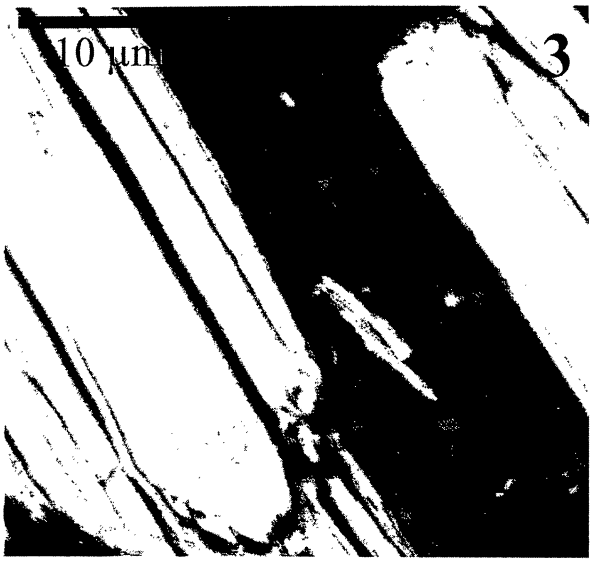
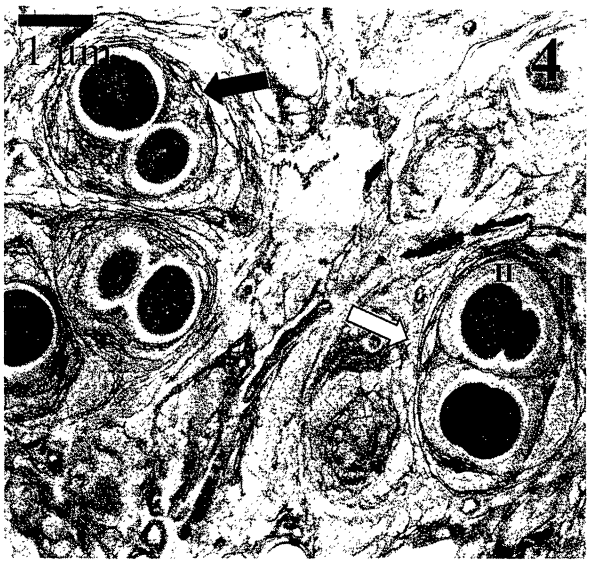
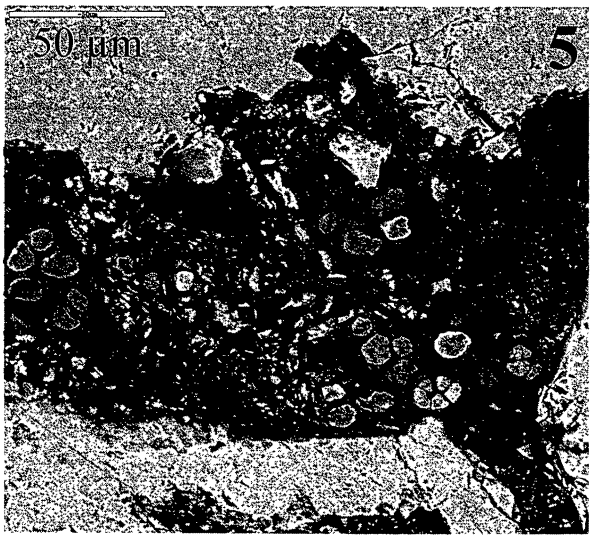
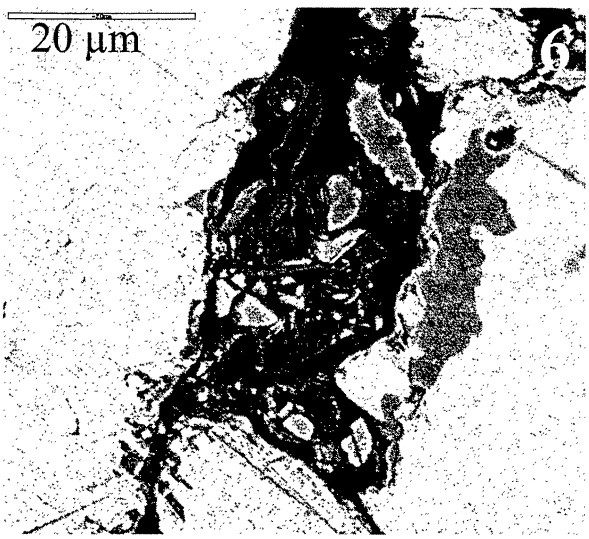


Fig. 2. SEM-BSE image of hypolithic cyanobacterial cells obtained by examining a fresh rock not embedded in resin.









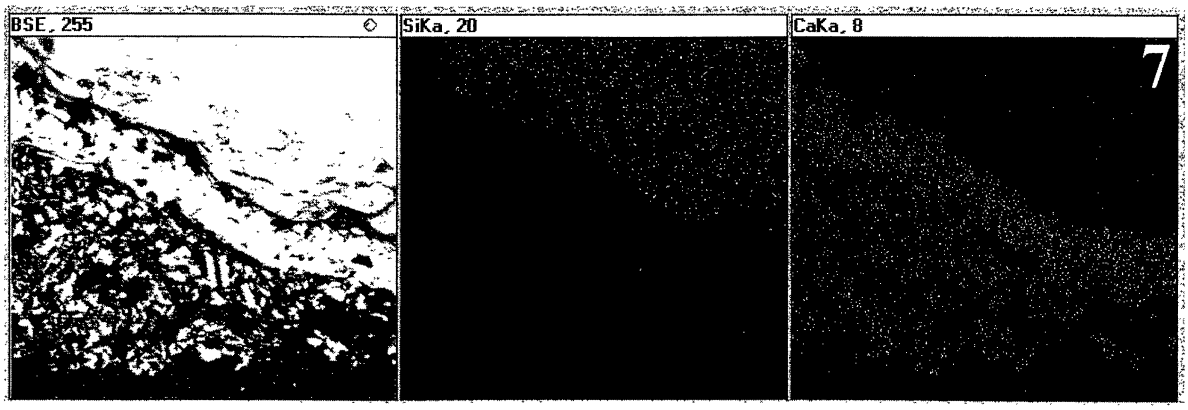


Fig. 3. SEM-BSE image of cyanobacterial cells occupying a near-surface fissure zone.

Fig. 4. TEM image showing cyanobacterial cells with different kind of sheath.

Fig. 5. SEM-BSE image of a fungal and bacterial endolithic community.

Fig. 6. SEM-BSE image of a fissure containing fungal cells surrounded by mineral deposits which could eventually became biomarkers.

Fig. 7. (A) SEM-BSE image of a fissured rock zone. (B and C) EDS spatial distribution of silicon (Si) and calcium (Ca) respectively.