Cytological Investigations of Lithobiontic Microorganisms in Granitic Rocks

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Abstract

This paper shows the ultrastructure of lithobiontic organisms in a granitic rock from the exterior to the interior, where fissures are found 1–2 mm from the surface. There is clear differentiation at cell level between mycobiont and photobiont cells located on the rock surface which forms part of the lichen thallus and predakaryotic and eukaryotic cells (algae and fungi) found in the fissures. As well as the observations at the ultrastructural level of the photobionts which live in fissures and cavities, immunolabelling techniques with colloidal gold have been applied to obtain an immunocytological analysis of Rubisco enzyme in some of the cells. The technique applied here permits Rubisco enzyme to be identified in algae-like cells belonging to the filamentous green algae and to be distinguished from bacteria. The photobionts found in fissures have been observed by transmission and scanning Electron Microscopy, observing the external characteristic surface of the algal cells. In 1976 Le Campion-Leanium used transmission Electron Microscopy (TEM) for the study of cyanobacteria from the ultrastructural level in a calcareous substrate, showing internal structures of the cyanobacterial cells such as thylakoids, polyhedral bodies, rodplasts, etc. Unfortunately the sample preparation technique based on double inclusion and thick replica of the samples in order to avoid photographic artefacts was used by freeing the mineral component, which could only be applied to calcareous substrates. On many occasions the biological material has been extracted from the substrate. In 1978 (Friedmann, 1982; Friedmann and Kortem, 1989, and processed later for Transmission Electron Microscopy). Very few investigations have been carried out at the ultrastructural level without extraction of the biological material from the substrate. TEM techniques were used for the “in situ” observation of the interface between the thallus of Lecanora albescens and a calcareous substrate (Ascasso and Olmstead, 1991). This technique encounters important difficulties even when applied to studies of the lichen thallus-substrate interface in soft rocks. This is why we used a technique of electron imaging technique applied to the study of the pelmatolichen lichens thallus-substrate interface was introduced recently (Vieruschek and Ascas, 1994) and may be applied to all types of rock substrates.

In the present work the lithobiontic organisms present in fissures of a granitic rock covered by Ascopila spec. lichen thallus were investigated at the cytological level using the transmission Electron Microscope. The immunolabelling of the cells located inside the fissures was also investigated by Energy Dispersive Spectroscopy (EDS).

Key words

Ascopila, granitic rock, immunogold labelling, microprobe, lichen, lithobiontic microorganisms, Rubisco.

Introduction

In 1981, Golubic et al. defined the lithobiontic term “lichenization”, organisms which live within hard mineral substrates. The physical and chemical characteristics of the substrate are related to the development of microorganisms. The hard rock substrates are very varied and bacteria live in them (including cyanobacteria), algae, fungi, lichens and bryophytes. The first cells that live in the rock are named chamaeclonialdium, cyanobacteria and xanthoria.

Materials and Methods

Granite rock fragments that show crustose lichen thallus of Ascopila on their surface were collected in Buxarresta da la Sierra, Madrid (Spain).

Once processed and prepared, as described in the following paragraphs, all the samples were investigated with SEM in back-scattered electrons (BSE) emission mode using the DMS 660-Zet-Ronaks microscope. The SEM was equipped with an EDS analyser.

Cytological study

Hand-cut pieces with corresponding thallus of lichen were processed according to conventional Transmission Electron Microscopy (TEM) procedures for the preparation of lichen material (Ascasso et al., 1968) applying the LR White resin as the electron conductive medium. The blocks were then cut transversely sections were prepared for cytological investigations by staining with lead citrate (Reynolds, 1963) for 10 min.

Immunocytological study

Immuno labelling was carried out with anti-Rubisco antiserum of Euglena gracilis, provided by Prof. Josipa Matic (Biochemistry Department, Valenciennes, France). The pieces were post stained with uranyl acetate followed by lead citrate (Reynolds, 1963).

Microprobe analysis

The microprobe analysis was carried out in the samples prepared for SEM observation and as in the samples prepared for immunolabelling. The microprobe analysis and spatial distribution of elements and microprobe profile was carried out with Energy Dispersive Spectrometry (EDS) Link ISIS micromaternal system.

Results

Figure 1 shows the general aspect of a granitic rock surface covered by the crustose lichen thallus of Ascopila. Figure 2 a detail of the zone marked by the arrow in Figure 1 and should be observed. Figure 3 shows the lichen thallus of Ascopila sp and the system of fissures found under the mineral situated beneath the thallus. The thallus displays an ovoidal shape with a celted, algal zone and an undifferentiated medulla. Fungal cells (black arrows) and algal cells (white arrows) can be seen in the fissures under the thallus. Figure 4 is an enlargement of the area indicated by a square in Figure 3. It shows two photobionts of thallus that are thallus with their central pyrenoids. The thallus is covered with a thin layer of pyrenoids completely filling the inside of the pyrenoids can be seen in the top right algal cell (arrows). The mycobiont cells present in the top left of the figure show numerous lipid bodies in the cytoplasm of the cells and the hand hypha displays a group of concentric bodies. Figure 5 is an enlargement of the area marked with the double white arrow in Figure 3. At the bottom of Figure 5 cells of microconfrus are shown and at the top, rounded algal-like cells. Figure 6 shows another nearby area within the same fragment of granite rock where a more complex system of fissures under the Ascopila thallus can be seen. Lithobiontic cells deeply penetrating into the fissures can be seen, in addition to lichen thallus lobes present at the top of...
Fig. 1. General aspect of a granitic rock surface covered by Aplidina s.p.

Fig. 2. Detail of zone marked by an arrow in Figure 1.

The fissure. The top fissure (F) lacks biological elements but the remaining fissures, two on the left of the figure, parallel to each other, and one on the right, are full of lithobiontic cells. Figure 7 is an enlargement of the area indicated by a single arrow in Figure 6. Looking at Figure 6, the reader may well imagine that the cells indicated by a single arrow are similar to the thallus cells or to the bottom fissure cells (shown in Figure 6 by a double arrow). Figure 7 shows that they are more likely to be cells of fungal origin. Figure 8 is an enlargement of these cells showing a large central lipid globule and smaller lipid droplets located in peripheral positions (arrows). Figure 9 is an enlargement of the area marked with two arrows in Figure 6. This image displays structures similar to Figure 7 despite its being in an area located much further inside the rock. Hyphae can be seen at the bottom of the fissure displaying a high lipid content in their cytoplasm whilst the top is formed by packed microfungal cells. Figure 10 relates to the left hand side of the fissure where Figure 9 is located. Cells can be seen which appear to be algal with peripheral lipid and a clear mass inside, which may relate to a more or less organized chloroplast. Fungal cells are clearly identifiable at the top left of this image.

Despite achievements in the ultrastructural observation of a fissure’s biological matter, it would also be advisable to apply immunohistochemical techniques which could demonstrate the presence of certain enzymes in cells inside the fissure. Figure 11 shows immunolabelling ofubisco by colloidal gold of 30 nm indicating the presence of this enzyme in the pyrenoid of an alga-like cell found in a fissure of an area near the lichen thallus location. Figure 12 is an enlargement of an algal pyrenoid. Figure 13 shows a fissure completely occupied by lithobiontic organisms. This fissure cuts through a micaceous mineral. Lithobiontic cells inside enclose sheets of mica. A similar situation is shown in the following figure (Figure 14) where a fissure crosses a micaceous mineral, where lithobiontic organisms and a fragment of the mineral can be seen separated from the top wall. This figure has a line drawn obliquely across it. The line represents a line scan for different elements as will be shown in Figure 16. Figure 15 shows, in the top left section, the same back-scattered electron image as in Figure 14 and the remaining three sections are EELS X-ray images showing the spatial distribution of Al, K and Fe on the bionite located at the top and bottom of the fissure. The Fe spatial distribution may exactly record the forms of the micaceous material shown in the BSE images. An evident lack of potassium in the bionite above and below the fissure was observed. Figure 16 shows microprobe line profiles across the line drawn in Figure 14 for Al, Si, K and Fe elements. The observation and subtraction of microprobe line profiles for Fe and for K precisely demonstrate sites of K release occurring on the bionite.

Fig. 3. General view of an Aplidina thallus and lithobionts situated in fissures under the lichen: al, algal cell; c, cortex; m, medulla. White arrows, algal cells; black arrows, fungal cells.

Fig. 4. Detail of algal layer marked in Figure 3. A, algal cell (photobiont); c, concentric bodies; H, hyphae (cytophory); L, lipid body; P, pyrenoid. White arrows, pycnocysts.

Fig. 5. Detail of fissure marked by two white arrows in Figure 3. A, algal-like cell; H, microutus.

Fig. 6. General view of a network of fissures under a lichen thallus (Aplidina). F, fissure. Arrows indicate the zones shown in Figures 7–8 and 9–10.

Fig. 7. Detail of the fissure marked by the top arrow shown in Figure 6.

Fig. 8. Enlargement of cells shown in Figure 7. L, lipid body. Arrows, small lipidic bodies.

Bar indicates 1 μm unless otherwise stated.

Figs. 3–8
Discussion

In 1987 Brock emphasized that for any study of microbial ecology one should be able to observe the organisms in their natural habitat. In the present investigation the use of SEM with a back-scattered electron detector to investigate polished samples of the thallus/rock interface allowed a clear differentiation at cellular level between mycobiont and photobiont located on the rock surface (Figure 4) and prokaryotic and eukaryotic cells (algae and fungi) found in the fissures. Figures 7 and 8 display cells identified as hyphae. These "in situ" observed hyphae look like the "oil hyphae" described in 1978 by Kausairi et al. who called such structures "oil hyphae" because they contained large amounts of lipids in the form of oil globules. Moderesti and Laljo (1988) observed that the hyphae forming bundles inside the substrate sometimes take on a globular appearance. Later, Garty (1992) observed externally some globules in fungal hyphae of an epilithic lichen growing in the bryozoan rock and also called these hyphae "oil hyphae". Probably the first observation of "oil hyphae" was by Bachmann (1904) who observed these structures beneath the thallus of Leccidae crustulata Ach.

Figure 9 of the fissure located 1 mm from the surface shows the same "oil hyphae" and other cells of rock-inhabiting microfungi. The presence of hyphae penetrating 2 mm into the substrate has been documented in Scanning Electron Microscopy working with secondary electrons in Lecanora muralis (Salazarori and Lazzarini, 1991). Rock-inhabiting microfungi are present in the enlarged fissure represented in Figure 5 and other types of cells can also be observed in this figure. They are difficult to identify at the moment, which shows that on occasions, certain biological components of the substrate are hard to identify from their ultrastructural appearance. This is why it becomes necessary to apply techniques to localize enzymes. Immunolocalizations, an example of which is presented in this paper, may clearly enhance "in situ" cytological studies of lithohiontic organisms. The technique presented in this work enabled Rubisco enzyme to be identified in algae-like cells belonging to fissures where it was not easy to ultrastructurally identify the pyrenoid of the cells, in order to know whether they were trebouxoid algal cells or not. Locating Rubisco in the photobiont of the thallus of Parmelia sulcata has been done in ultrathin section in TEM (Ascanio et al., 1995) and the present work confirms the existence of algae of a trebouxoid nature in
Figs. 13 and 14 shows a biotox containing lithobiontic and lithobiontic organisms. Dons and Carney (1990) observed that lithobionts develop along the calcareous rock crystals destralying the coherence of the crystal to the rock. Lithobiontic organisms (Golish, et al., 1981) are dead endoskeleton when microorganisms actively penetrate calcareous substrates in the microcosms shown in Figs. 13 and 14, the cells pass through previously well-picked micas which currently appear poor in K. It can be clearly seen that the mica types of granite ducts can be distinguished by bell et al. (1994), several took place in the microcosm shown in the figures. Physical disruption of minerals or uptake of mineral elements. In this case, potassium, but we cannot identify other elements. This loss of potassium from the micas shown in the mapping of Figure 15 demonstrates an alteration of the mineral substrate in this reproductive element. The litholamellae itself appear to be chalcedony, silicates, carbonate minerals, and their crystal fragments can be clearly seen as deformed endosomes. The loss of potassium from the micas may be attributed to oxalic acid being secreted by phytophotos, since Eckardt (1998) experimentally demonstrated a heavy extraction of K over a period of several days due to the action of M oxalic acid. Solubilization processes in which bacteria and fungi are involved, with dissolution being performed by acids or complexing agents have been well documented. For example, Robert and Berthelin (1986). Acsani and Wierzchosziewicz (1994) showed algal and fungal cells closely packed within micas sheets occupying a semi-transparent position in the rock. Surface located micas could also be seen to undergo losses of K when in contact with lichen thallus cells (Wierzchosiewicz and Acsani, 1995). The possibility low in the thallus lichen facilitates K’ protonation and washing away from interlamellar areas. Although some papers on the influence of calcium and magnesium on the growth of some marine species on slate exist (Armstrong, 1990). However, the destination of K and its role, if this element is indeed taken up by the thallus, is practically unknown. The few research papers existing on the subject state that this element is preferably located in the algal area (Asta, 1993) whilst Ga’ is located in the mineral tissues.

The evidence of K’ losses in micas belonging to feldspars, as observed in “zita” in this paper, and the evidence of K’ losses in surface micas where rhizines of Parmelia caperata is attached (Wierzchosiewicz and Acsani, 1995) provide support to account for the phenomena occurring in microsites. The capacity of lithobiontic organisms to act on the mineral material nearest to their walls could clarify aspects relating to the biodegradation caused by epilithic and endolithical organisms. Acknowledgements

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References


