main uncertainties concerning the structure and the chemical composition of the Zn-rich deposits. Both inconsistencies concerning the crystalline and non-crystalline structure of deposits with similar element composition in Platanis and the occurrence of Zn-rich crystals without a significant P content but a relatively high S peak (Fig. 11d) suggest that there may be several storage forms for Zn in Thaspai. But as the resin used for sample preparation in this study contained S and background values for S in EDAX spectra were high, further investigations are required to confirm the hypothesis that S-containing compounds such as gluconolactone are involved in Zn tolerance in Cruciferae (Matsuy, 1977). Moreover, EDAX is not suitable for detecting elements with atomic numbers lower than eleven, so that the proportion of C, O, N and P that may be present in the Zn crystals cannot be verified by this technique. Investigations of these crystals by X-ray microdiffraction techniques would be helpful for further characterizing the Zn storage form in vacuoles of Thaspai.

Acknowledgements

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Abstract

The profound knowledge of the structural and chemical characteristics of the interface between lichen thallus and rock, seems to be indispensable for the process of understanding the lichen symbiosis as well as the significance of the weathering action of lichens. One of the most promising techniques to be used in this investigation is the scanning Electron Microscopy of SEM (behaviour) in the back-scattered electron mode (SEB) emission mode. In the present study on thallus of Parmelia conspersa, Aspicilia intermutans and Lecidea aculeata growing in granitic rock were examined by SEM in SEB mode with Energy Dispersive Spectroscopy (EDS). In the study the observation of the interface permits detection of the rhizoids/hyphae adherence and determination of the origin of the minerals which adhere to the rhizoids/hyphae. In the case of the crustose thallus SEM permits investigation within the structure of the crustose thallus and crustose lichen-rock contact zone and also allows observations on the penetration and filling of the fissures and cracks of the underlying rock by components of the thallus and other living organisms. The ESB images can contribute to a better knowledge of the cytological state of the rock-inhabiting organisms and also to the understanding of the action of the chemical treatments used in the removal of lichen from building materials.

Keywords

Pormelia conspersa, Lecidea auriculate, Aspicilia intermutans, back-scattered electron imaging, lichen, rock, weathering.

Introduction

The study of the lichen-thallus-rock substrate interface was initiated many years ago with the application of various techniques. Special emphasis has been placed on the necessity to investigate the type of biogeophysical or biogeochemical action that could occur under a determined lichen-species and the material obtained was used to study the particular techniques for the longest time was Light Microscopy (LM) and it provided a view of the thallus-substrate relationship and also knowledge of some minerals present in the rock. However, the low resolution of light microscopy in showing the living elements of the thallus and identifying the microcrystals present immediately below the thallus made it a limited technique.

From 1968 scanning techniques were used to obtain material from the lichen-rock interface and the material obtained was studied with the usual techniques of clay mineral research. The first investigations in this field were carried out by Daffner (1968) and Ascaso et al. (1976). They showed that minerals appeared in the interface zone that were not present in the underlying rock.

In 1977 Hallbäser and Jahns observed the interface zone with new techniques that permitted better resolution than the light microscopical techniques used before. These authors combined, for the first time, Scanning Electron Microscopy and Energy Dispersive Spectroscopy (EDS) to distinguish the difference between the lichen and inorganic parts.

Between 1980 and 1982 various work appeared which combined L. M. with scanning techniques. With the latter a mixture of biological material of thallus and rock mixture of biological material of thallus and rock mineral were obtained. This mixture was treated for various times with belling H2O2 which removed the biological material. The remaining inorganic material was investigated with the usual technique of clay mineralogy, such as Infrared Spectroscopy (IR), X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM).

Although with these experiments comprehension about the mineralogy of the interface has improved, there has been a growing interest amongst in-
vestigators in finding less destructive techniques for investigating the mineralogy of the rock surface under the thallus and also to know the relationship between the symbionts of the thallus and the mineral elements of the substrate. With this in mind, since 1985 there have been many projects which combined LM and SEM techniques (on occasions with EDS). In the case of Ascua and Wierzchos (1994) for a review, unfortunately the biological and mineral materials are non-conductive substrates which require coating with a layer of heavy metal for study with SEM techniques in secondary emission mode. The heavy metal coatings render the specimens unsuitable for EDS analysis. Moreover the topographic images of the contact zone can contain artifacts resulting from the detachment of small particles, or these particles can be obstructed by biological material. The first attempt to apply back-scattered electron images (BSE) to the lichen-rock interface was reported by Chisholm et al. (1987) and Forveille et al. (1987, 1990). However, in their BSE images a significantly brighter response sufficient for a microtopographical and ultrastructural study was not obtained, probably due to the preparative procedure.

In the past few years a new objective has appeared in this kind of investigation, that is, to work with techniques that provide the capacity to understand the ultrastructure of lichen symbionts lying close to the rock surface. In 1992 ultrathin sections of the lichen-rock contact zone were investigated by Transmission Electron Microscopy (TEM) (Ascua and Oinasartjota, 1993) to achieve a better knowledge of the relationship between the thalli of the lichen thallus and the adjacent minerals. These observations also facilitated the investigation of the mycobiont ultrastructure and also the physicochemical ultrastructure. In the same research the use of EDS permitted a better understanding of the chemical composition of different bodies of the rock minerals situated inside the thallus near the symbionts. Although this method is very thorough, in the study of the interface of crustaceous lichens it presents some limitations. One of them is the great difficulty in obtaining ultrathin sections of the lichen rock interface, another is that the observation area of the interface is reduced to 1–2 mm². Taking into account the limitations of the above-mentioned techniques, the SEM with ESE and EDS seems to be a very adequate and complementary tool in the study of the lichen-rock interface. The application of this technique fulfills the following prerequisites: a) wide scope of observation, preferably of several cm² as used in LM, b) high resolution power close to that of TEM, c) possibility to carry out a chemical analysis of inorganic compounds "in situ".

In the present work the application of SEM with ESE and EDS provided knowledge of new aspects of lichen symbiosis and its relationship with the substrate, giving answers to the mineralogical aspects of the interface as well as to the existence or non-existence of cross contamination. It is also possible to clear doubts about the living elements existed in the existing fissures below the thallus and improve the observation of ultrastructural aspects of mycobiont and photobiont.

Materials and Methods

The thallus of Parmelia conspersa (Eleophyllum sect. "Par-lepsia"). Lecidea auriculata Th. Fr. and Aspicilia intermedia (Nyl.) lichens growing on granitic rock at Bustarviejo de la Sierra, near Madrid (Spain) were collected. A previous photomicrography of this material showed the presence of quartz, orthoclase, and plagioclase in granitic rock as essential minerals, and biotite (muscovite mineral), zircon and apatite as accessory minerals (Ascua, 1985). Hand-cut pieces of rock with corresponding thallus of lichen were processed according to conventional TEM procedures for the preparation of lichen material (Ascua et al., 1986) applying as the inclusion medium, the 1.1 M lithium picrate resins. After polymerisation the block was sawn in the transversal direction. Afterwards the obtained surfaces were hand polished with abrasive powders of decreasing size. Then the fine polished surfaces (powder diamond grain diameter 0.25 µm in the polishing) were stained with lead citrate (Reynolds, 1963) and, after washing in distilled water and air drying, the surfaces were coated with evaporated carbon. Detailed descriptions of the preparation of transverse sections of the lichen-rock interface was given by Wierzchos and Ascua (1994). The samples were then examined with SEM Zeiss 900 EDS equipped with a BSE detector and EDS Link ISIS microanalytical system.

Results and Discussion

The Parmelia conspersa lichen thallus is attached to the substrate by rhizines, as shown in a transverse section of the lichen-rock interface in Figure 1. In the zone where the rhizines are adhered to the rock it is possible to observe a mixture of hyphae belonging to the thallus and minerals (arrow). A more detailed observation of the lower part of the rhizine (Fig. 2) demonstrates how the hyphae detach the rock minerals by mechanical action. These mineral particles demonstrate the same geologic nature as the part of the rock belonging to the substrate. Nevertheless the observation of the contact zone also shows mineral particles which were deposited in the biological part by weather factors such as rain and/or wind. From the earliest mineralogical research on the interphase zone (Ascua et al., 1976) the question of the origin of these minerals had great scientific interest. The arrows note two

Fig. 1. Transverse section of Parmelia conspersa interface with granitic rock. Arrow indicates minerals attached to the rhizine, T, thallus; R, rhizine.

Fig. 2. Detail of the lower part of rhizine shown in Figure 1. The lower arrow indicates a piece of mineral inside the rock. The upper arrow indicates a fragment of the same mineral.

Fig. 3. Detail of Figure 1. Spherical body with aspect of soredium. A, algae; Arrows, pyrolaids.

Fig. 4. Transverse section of Lecidea auriculata growing on granitic rock. Union of thallus (T) and mica mineral (M).

Fig. 5. Detail of Figure 4. A, algae; C, cortex; H, hyphae; M, mica layer.

Fig. 6. Detail of the arrow-noted part in Figure 4. A, algae; M, mica.

Fig. 7. a: general view of rock surface and fissure (arrow), b, interior of figure. Wide arrow, algal cell; little arrow, lipid bodies.

Fig. 8. High magnification of algal cell and hyphae. C, chloroplast; H, hyphae; P, pyroplaid. Arrows, lipid bodies.
parts from the same mineral, one belonging to the rock and another embedded between the hyphae of the thallus. The picture shows the size and distribution of these minerals and also permits observation of the anatomy of the thallus. On the left side of the figure it is possible to distinguish a spherical body. This spherical body is shown in higher magnification in the next figure (Fig. 3). The algal cells are clearly shown. Part of the fine structure of the algae is also shown, particularly the pyrenoid (arrows). The algal cells are mixed with fungal cells giving a structure with the appearance of an aerodium. This structure lies attached to different microdivided minerals.

The observation of the *Leccidea auriculata* interface has elements of great interest. Fig. 4 shows all aspects of the contact between the thallus of *L. auriculata* and the micaceous substrate. The plates of mica are becoming more and more broken. The next BSE image (Fig. 5) shows that algae, which appear in the center of the figure bonded close to mica sheets, have a central chloroplast with a pyrenoid which is typical for trebouxoid cells. The algal cells seen in Fig. 5 are clearly different from the algal cells shown in a deeper part of the contact zone (Fig. 6 proceeds from the plate marked by arrow in Fig. 4). Probably, the observed algal cells in Fig. 6 are free-living algae. More investigations must be done with SEM-BSE to better understand the infrastructure of these algae. In this part of the micaceous mineral (Fig. 6) the plates of mica are not yet separated. However, groups of algae are present, even inside fissures as narrow as 10 μm diameter.

Another aspect of the lichen-rock interface, is given in Figure 7. In Figure 7 a a general view of the rock surface is presented. The wide arrow indicates the presence of biological material inside a fissure. In Figure 7 b a highly magnified image of the fissure is presented. It is possible to identify algal cells (arrows) with an abundant presence of lipid bodies inside the cytoplasm (little arrow). The origin of these algal cells is unknown, since they may or may not belong to the lichen thallus. In a high resolution image (Fig. 8) the contrast has been inverted electronically, and this inverted contrast BSE image is comparable to that of the TEM. Note that chloroplast and pyrenoid are distinguishable in algal cells. The fungal cells showed dark zones which correspond to lipid bodies.

Near the *L. auriculata* thallus it is possible to find structures with aspects of cells mixed with inorganic crystals (Fig. 9). Observation at higher magnification shows that the above-mentioned cells are hyphae (Fig. 10). The analysis by EDS has shown that the crystals are calcium oxalate. These crystals of calcium oxalate remain intercalated with minerals detached from the rock. The presence of calcium oxalate in lichens in the form of plates of monohydrate calcium oxide or bipyramids of dihydrate calcium oxide has been confirmed in numerous publications (Ascenso et al., 1982; Nimis et al., 1992). Recently SEM with EDS has allowed observations of the typical morphology of these oxalates and, at the same time, the analysis of their chemical composition (Adamo et al., 1989; Adamo et al., 1992). Canini et al. (1991) showed a polished cross-section of lichen-rock interface with the distribution (mappng) of Ca⁺ in the lichen thallus. Edwards et al. (1993) had demonstrated, using FT-Raman Microscopy, that hyphae, calcium oxalate and decomposing products are present up to 20 mm into the stonework.

Figure 11 shows the interface of the *Aspilia intermutans* thallus. The algal layer, as well as the cortex and medulla, are easily identifiable (black arrows). The figure also shows, on its left side, a piece of mineral. The lower part of the piece has the same shape as the rock-mineral situated immediately under it (white arrows). Between the piece of mineral already mentioned and the rock minerall there exists a fragment of a well-developed thallus. In a zone situated very close to this (Fig. 12) several plates of micaceous material (arrows) appear embedded among the hyphae of the thallus. These plates seem to be detached from a micaceous material situated on A in the figure. The application of the EDS microanalytical system to study detached minerals allows the definition of their chemical nature.

Apart from the importance of the study of the thallus anatomy with several details of the fine structure of the symbionts, and the interest in knowing the aspect of the rock surface which is in contact with the thallus, the investigation of the fissures situated below a determined thallus are also a subject of interest. Figure 13 shows a piece of lichen thallus and also several fissures situated inside the rock (arrows). The aspect of the biological material present inside the fissure is shown in Figure 14. The algal cells are presented in Trebouxoid aspect with several lipid bodies in the cytoplasm. Going to the interior of the fissures it is possible to observe algal cells situated several mm from the surface. Note that algae in cell division processes were observed (Fig. 15). In a very close superficial zone there are hyphae whose cellular structures can be appreciated at high magnifications (Fig. 16).

Although the principal aim of our work was the study of the interaction between lichen thallus and rock, the observation of rock surface with no lichen cover was also done. In this zone the lack of characteristic elements of bioweathering processes was detected.
“Pollen Buds” in Ophiorrhiza (Rubiaceae) and their Role in Pollenkitt Release

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Abstract

Pollen buds are apertural protrusions formed by the ectine. In their wall unsterilized and methyl-sulfonated pectins are detected by immunogold labeling. During pollenkitt formation pollen buds become depolarized and connected to the tapetum wall. The following expansion of the endothecium causes the mechanical rupture of the tapetum at the binding sites of the pollen buds. These events are accompanied by the release of the pollenkitt.

Key words
Apertural protrusions, ectine, endothecium expansion, pectins, pollenkitt transfer, tapetum.

Abbreviations and Symbols

- pb: pollen bud
- el: endothelial layer
- m: middle layer
- rk: pollenkitt
- t: tapetum
- TEM: transmission electron microscopy
- SEM: scanning electron microscopy
- LM: light microscopy

Introduction

Pollen buds are defined as apertural intrusive protrusions and were first reported by Philip and Mathew (1975). In the formation of pollenkitt the pollen buds and their detachment from the pollen grain have been recorded from various taxa of Rubiaceae (Cheninneaeastrum and Scleranthus, 1983; Mathew and Philip, 1987; Igersheim and Weber, 1993). The essential point in the research of Philip and Mathew (1975) and Mathew and Philip (1987) was that the vegetative nucleus is eliminated from the pollen grain via pollen bud detachment. Igersheim and Weber (1993) refute the published data in their ultrastructural re-investigation: the vegetative nucleus remains in the pollen grain even after the pollen buds are detached.

The present review is now focused on the functional aspects of pollen bud formation and their detachment, and explains a possible role in pollenkitt release.

Materials and Methods

Authors

- The heterogeneous Ophiorrhiza longifolia (R.) from Java, Puff 290516-1/4 (Figs. 1-2, 14-17) and of an autogamous Ophiorrhiza* species from Sarawak, cultivated in the greenhouses of the Botanical Garden of the University of Vienna (cult. HIR sub No. 89-7, coll. M. Fritze, a. n. (Figs. 3-13)) were investigated.

For LM and SEM, authors were glued in BPA. Microme techniques used for material embedded in KUL3511 Zechertchnov 7100 follows Igersheim (1993). Pollenkit lipids were stained with Sudan black (0.08% for 5 min, stained with glycine and distilled water (1:1), and embedded in glycerine jelly. Pollen were localized with Ruthenium red staining (Geilich, 1984). For contrast enhancement Ruthenium red staining was post-stained by toluidine blue (Figs. 1-2).

For SEM investigations authors were dehydrated in an alcohol series and FDA Giesterberger and Leins, 1973) and subsequently critical-point dried. The specimens were mounted on aluminum stubs with nail polish and sputter coated with gold.

The TEM embedding and staining processes followed Weber (1993).

For immunolabelling Oto, post fixation was omitted. After dehydration in a graded alcohol series samples were embedded in EMBED 812 (London Resin Co., Ltd). Ultrathin sections were collected on gold grids and treated as follows: Pre-infiltration in 2% buffered BSA solution (0.1 M Tris-HCl 7.4 and 0.1% Tween 20) for 30 minutes at room temperature; 2% infiltration with the primary antibody (JMS or JMT (produced in rabbit which recognizes unesterified JMS and methyl-esterified JMT (JMS) pectins (Knox et al., 1996). After washing in PBS, sections were floated on a 3% solution of goat anti-rabbit IgG coupled to 10 nm col-