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Some Initial Stages in the Formation of Epilithic Crustose Lichens in Nature: a SEM Study

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Abstract

The formation of new lichen thalli under natural conditions has never been observed in epilithic crustose lichens. In the present study we observed under natural conditions, free-living cells of green algae, probably *Trebouxia*, which is a common photobiont in lichens, and ascospores produced and discharged by the mycobiont of the lichen *Caloplaca aurantia.* Contacts between germinating lichen ascospores and algal cells take place inside pits and ruts on old, weathered concrete roof tiles. During the early stages of formation of this epilithic crustose lichen, dust particles are entrapped in the young lichen thallus.

Keywords: *Trebouxia*, free-living photobiont cells, ascospores, lichen, *Caloplaca aurantia*

1. Introduction

In epiphytic lichens it was shown (Jahns et al., 1979) that algal cells form clumps that are intimately associated with long fungal hyphae, the latter probably deriving from spores of the lichen *Lecanora varia*, dispersed on needles of the tree *Picea abies.* Recently, germinated spores of the epiphytic foliose lichen *Xanthoria parietina* have been observed on tree bark and, in some cases, germinated spores were found in close proximity to *Trebouxia* and/or *Pseudotrebouxia* cells (Bubrick et al., 1984).

In the present study we wished first to determine whether unicellular green algae, which serve usually as photobionts in epilithic crustose lichens, may live also in free colonies.

A further goal was to ascertain whether the free spores of a lichen growing on the same substrate and producing fruiting bodies can be found in the vicinity of free algal cells. We also hoped to discover sites on the substrate surface where active contacts between algal cells and germinating spores of the mycobiont may take place. The underlying purpose here was to observe some of the early stages of lichenization and the formation of epilithic crustose lichen thalli in nature. To obtain these goals the following conditions were requisite: (a) the substrate needed to be covered chiefly by a single lichen species producing numerous fruiting bodies but no vegetative diaspores; (b) this lichen, a crustose one, had to occur both in young and old colonies, so as to provide information about the vitality of the biological population; (c) the lichen coverage on the substrate had to be less than complete, thus enabling easy sampling of bare areas on which lichenization may occur.

2. Materials and Methods

The crustose lichen *Caloplaca aurantia* (Pers.) Hellb. var. *aurantia* was chosen because it is very common and abundant in Israel (Galun, 1970). This lichen does not produce any vegetative diaspores. Observation, focused on the upper surface of concrete roof tiles taken from an old house in the village Ganne Am, 20 km NE of Tel Aviv. Sample tiles were finely fractured to yield bare debris, which were fixed overnight with 3% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.4, washed with the same buffer, dehydrated in increasing concentrations of ethanol, dried with a critical point drier and finally coated with gold. The samples were examined by a Jeol-35 scanning electron microscope (SEM) operating at 25 KV.

3. Results

The bare debris from the upper surfaces of the sampled tiles presents the marks of two patterns of weathering: pits and ruts (Fig. 1). The unweathered portions of the tile however, remain smooth and are not pitted. Where pits and ruts are evident, many of these are colonized mainly by one kind of unicellular green alga, presumably *Trebouxia*. Specimens of this alga are mostly 7–8μm in diameter and occur generally in aggregates of several, or even dozens of cells. The inner margins of the pits and ruts are smooth, due to abrading chemical action of the algal lithobionts on the carbonates of the concrete.

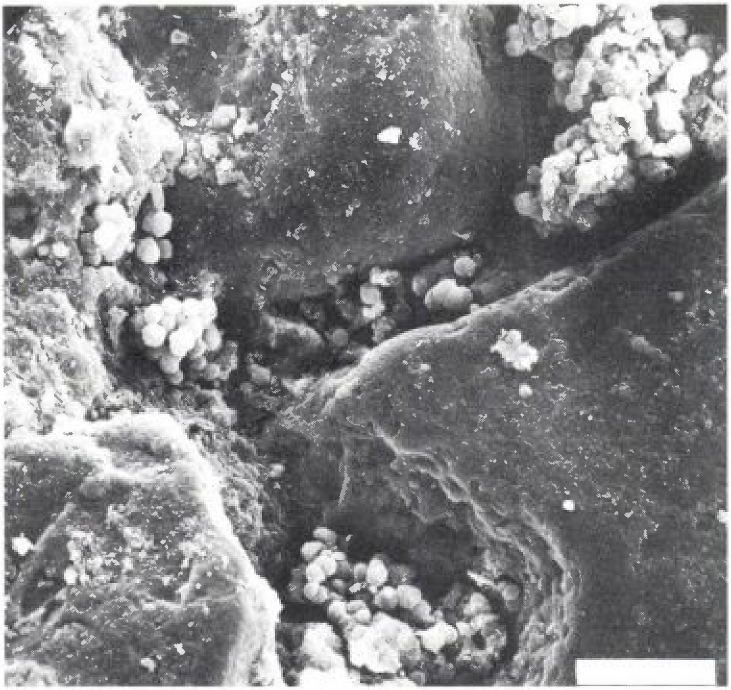


Figure 1. Upper surface of a seemingly bare part of a 60 year-old concrete roof tile. The weathered parts of the tile are pitted and colonized by a green unicellular alga, presumably *Trebouxia*. The inner edges of the pits are smoothed by chemical action of the algae on the carbonatic components of the concrete. The same algae also colonize ruts. The parts of the tile surface which are not weathered are relatively smooth. Bar = 50μm.

The established specimens of *C. aurantia* bear numerous apothecia, which produce large numbers of fungal (mycobiontic) ascospores, measuring about 10μm in length. These spores are entrapped in the pits and ruts (Fig. 2a). Such ascospores are easily identified because they are placodiomorphic and their size and shape conform to those usually given for this lichen species (Galun, 1970). When properly photographed in SEM such spores display a darker zone at each pole. An enlargement of Fig. 2a is given as Fig. 2b, showing that five of the ascospores presented (arrows) have shrunken walls. These ascospores seem to be older than the other ascospores which are shown in Fig. 2b and which were produced probably by different asci. Another epilithic lichen abundant in the same situation is *C. citrina*, but this lichen colonizes mainly asbestos roof surfaces and its apothecia are rare (Galun, 1970). Thus, ascospores shown in Figs. 2a,b are very probably from *C. aurantia* and not *C. citrina*.

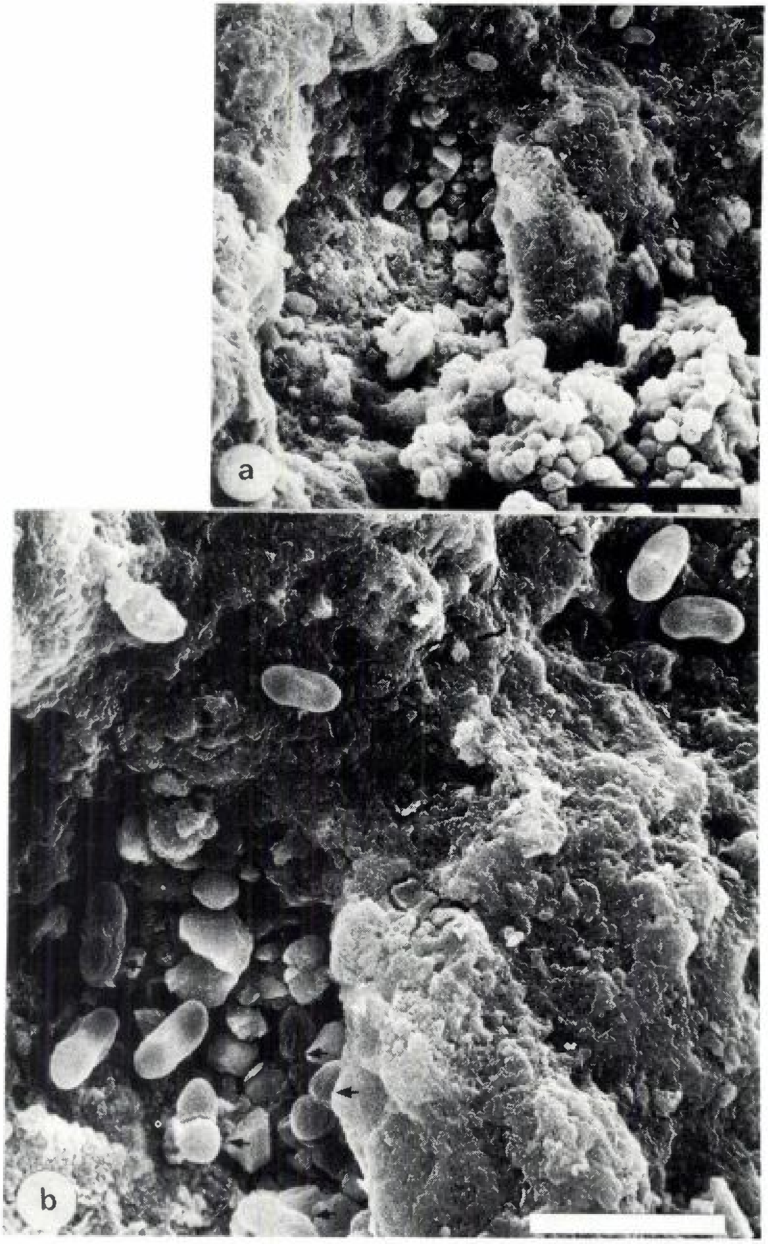


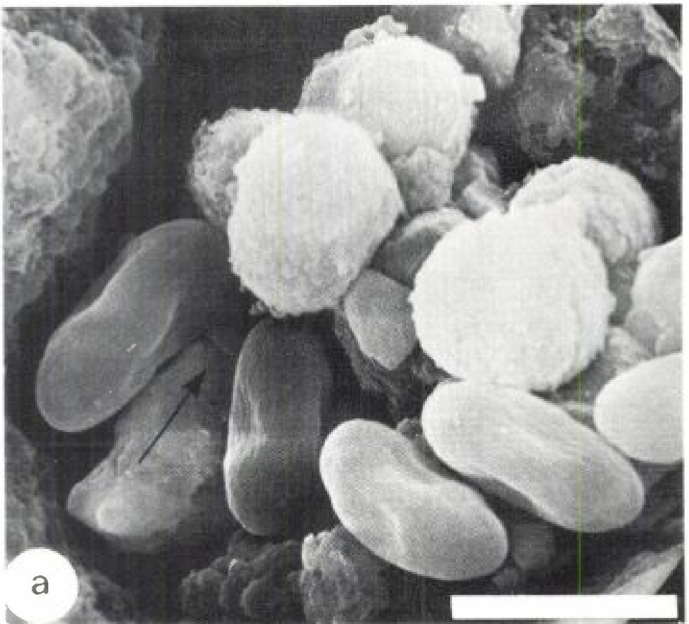
Figure 2a. Free photobiont cells and mycobiont spores inside a large pit on the surface of concrete tile debris. Shape and size of the spores fit those of the epilithic lichen dominating the roof tiles. The spores are placodiomorph, a typical feature of *C. aurantia* spores. Note the darker zones at the spore poles. Bar = $50 \mu m$.

Figure 2b. Enlargement of Fig. 2a, showing five ascospores with shrunken cell walls. These ascospores (arrows) are older than the six others shown in the picture. Bar = $20 \mu m$.

Once the spores have dispersed in the appropriate pits, they germinate under favourable moisture conditions. The spore on the bare debris collected during the winter of 1986 were easily detectable during the germinative phase by their germinating tubes which extend out of the spore (Fig. 3e). In many cases *C. aurantia* ascospores were found to germinate close to the algal (*Trebouxia*) cells (Fig. 3a). During germination the mycobiont hyphae may expand beyond the limits of the substrate cavities. On this lithic substrate, the mycobiont hyphae encounter dust particles (Fig. 3b). In the course of thallus formation, dust particles become incorporated in the lichen structure; Fig. 3b shows such a network of mycobiont, photobiont and dust particles. After establishment of the medulla in the young thallus and after the formation of the algal layer, the stage of cortex formation takes place. Because investigation of the latter two stages was not the main goal of this study we merely note in passing, that during the early phases of the cortex formation it is still possible for airborne dust particles to become entrapped by the young thallus, inasmuch as the upper surface of the lichen is still mostly unsheltered (Fig. 4a). At a later stage the fully developed cortex (Fig. 4b) more efficiently protects the inner parts of the crustose thallus from the enchroachment of deposited airborne particles.

4. Discussion

Free-living photobionts could be microcolonies derived from zoospores of algae within existing lichenized associations (Ahmadjian, 1980). According to Hawksworth and Hill (1984) photobiont cells may be acquired: (a) from free-living algae; (b) from existing thalli of other lichens or (c) from the vegetative propagules (e.g. soredia) of other lichens. Only few publications have reported the observation of free colonies of *Trebouxia* (Bubrick et al., 1984; Nakano, 1971a; 1971b; Tschermak-Woess, 1978). Algal symbionts such as *Myrmecia* and *Trebouxia* were often found near the lichen thalli (Tschermak-Woess, 1978). Alga, fungi and bacteria have also been reported in concavities of the very young needle epidermis and around the stomata of *Picea* trees (Steffens, 1986). Pits and ruts seem to provide important means of trapping algae and spores on sloping or horizontal lithic parts of buildings which are devoid of organic adhesive resins. The inner margins of the pits and ruts are smooth, due to the abrading chemical action of the algal lithobionts on the carbonates in the concrete. In a roof-slope situation this is especially important and during the run-off of rainwater from the roof-tile surfaces, the cavities containing the algae and/or lichens ascospores serve also as traps for



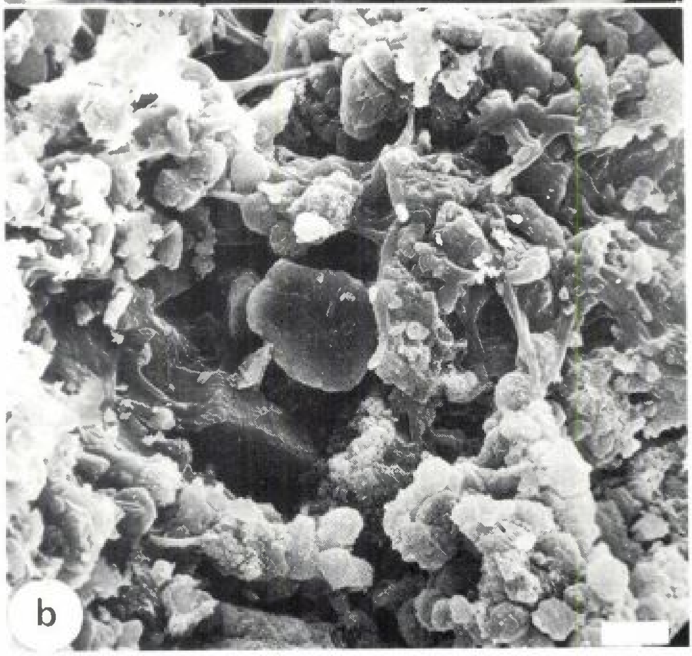


Figure 3a. Ascospores of *C. aurantia* in close contact with spherical cells of the photobiont *Trebouxia*. Note a germination of one of the ascospores (arrow). Bar = 10μm.

Figure 3b. During the establishment of the epilithic crustose lichen thallus, dust particles in a variety of shapes and sizes are entrapped by the mycobiont mycelium and are subsequently incorporated within the photobiont-mycobiont association. Bar = 10μm.

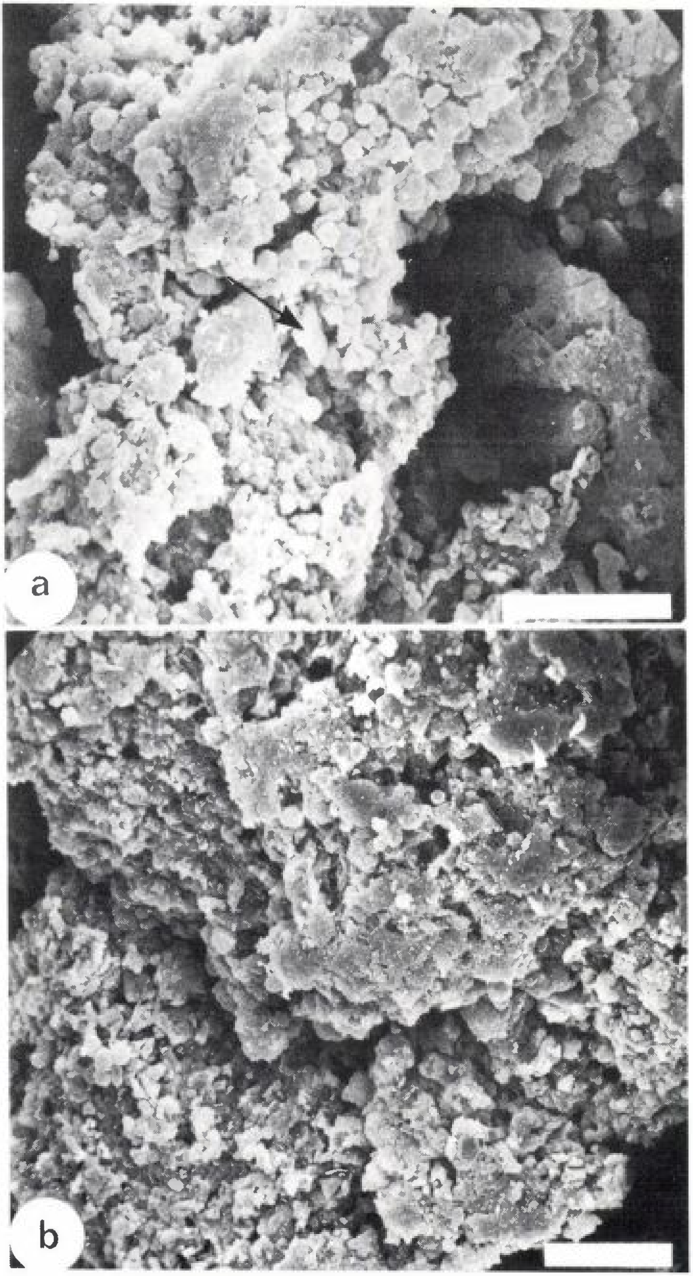


Figure 4a. Early phase of cortex formation in the lichen *C. aurantia*, showing the algal layer and the initiation of "carpeting" of the photobionts by cortical fungal hyphae. Note the airborne dust particle (arrow) on the algal layer. Bar = 50μm.

Figure 4b. Cortex formation at a more advanced stage. Algal cells are now hard to identify. Bar = 50μm.

moisture. Pits and ruts may act as traps also for airborne particles. Such particles are shown in Figs. 3b, 4a,b in this study and were found previously (Garty et al., 1979) in close association with the mycobiont medullary hyphae of mature *C. aurantia* growing at the very same site. These were shown to be composed of quartz, calcite, magnetite, alkaline amphibole or metallic iron latticed with titanium.

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